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Pesticide Background Statements

Volume IV. Insecticides

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Pesticide Background Statements

Volume IV. Insecticides

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This document reports the results of research only. Mention of a particular pesticide does not constitute a recommendation for use by the U.S. Department of Agriculture nor does it imply registration under FIFRA, as amended.

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1.0 Introduction

This section provides general information relevant to all of the Insecticide Background Statements. It primarily addresses those factors that are important to an understanding of the technical information presented in each background statement. General information concerning toxicity testing procedures, rating of relative toxicities, environmental fate and transport of pesticides, and assessment of hazards is presented as background for the discussion of specific material in the background statements for each insecticide. For convenience, a table of useful conversion factors and a glossary of terms and definitions have been included as sections 5.0 and 6.0, respectively.

The individual Insecticide Background Statements have been compiled to provide a comprehensive review of the available information concerning the use, chemistry, toxicology, environmental fate, and comparative hazard in forest applications. It is not within the scope of the Insecticide Background Statements to critically evaluate and/or independently analyze the raw data from which conclusions were reached in the various studies and reports examined in preparing the background statements contained in this document. References to the published literature at the end of each background statement are provided for those individuals who wish to independently evaluate the toxicological data and environmental fate information that is presented in summary form. In many instances, secondary sources, such as review articles, handbooks, and company technical data sheets, were used. Wherever possible in these instances, the primary source was also indicated and referenced, although it may not have been examined.

1.1 Insecticide Use by the USDA Forest Service

Management programs are used by the USDA Forest Service for control of insect pests and noxious and poisonous plants; wildlife habitat improvement; domestic range revegetation; maintenance of forest improvements; site preparation; conifer release; thinning timber stands; and control of disease and insects in nurseries. A number of means are used for management; including fire; mechanical methods; manual methods; biological control; and biological and chemical pesticides. These means of control are often used in combination rather than alone, and they vary in effectiveness and utility with factors such as vegetation, topography, proximity of inhabited areas, and competing management objectives (U.S. Department of Agriculture 1978).

A wide variety of chemicals that show pesticidal activity are available to land managers. Most pesticides are originally produced as water-insoluble crystalline acids or powders that are processed to form more soluble compounds (e.g., salts) or to produce formulations (e.g., emulsions) that can be readily applied to, and taken up by, plants.

The quantities of pesticides applied depend upon the time of year, type of vegetation, specific management objectives, and the properties of the pesticide. Rates of application are generally stated in terms of pounds of acid equivalent (a.e.) or pounds of active ingredient (a.i.) applied per acre, depending on the specific chemical nature of the pesticide formulation and carrier (U.S. Department of Agriculture 1978).

1.2 Physical and Chemical Properties

A number of physical and chemical properties of the pesticide active ingredient, carrier, surfactant, or other adjuvants ultimately determine the behavior of the chemical in the environment. For instance, cationic (positively charged) pesticides tend to be readily adsorbed by colloidal soil particles and thus become immobilized. Anionic (negatively charged) and nonionic (neutral) pesticides are more mobile in the soil since they do not tend to adsorb to soil particles. Water solubility of pesticides also affects their mobility--chemicals with a low water solubility (i.e., less than 1 ppm) tend to be relatively immobile, while chemicals with a high water solubility tend to be more readily leached out of soils, particularly if they also do not bind tightly to soil. The vapor pressure of a pesticide affects its volatility and thus its ability to dissipate through the atmosphere. The degradability of a pesticide by sunlight or by chemical or biological activity determines its persistence in the environment. Finally, the biological properties of a pesticide, such as its mode of action and its biological storage (bioaccumulation) properties, determine its effect on nontarget organisms (Weber 1977).

Each of the individual Insecticide Background Statements briefly presents information on normal use patterns and application rates, particularly as used by the USDA Forest Service. In addition, a summary of available data on chemical and physical properties is provided, with emphasis on parameters such as solubility in water, solubility in organic solvents, and vapor pressure, all of which influence behavior in the environment.

2.0 Toxicity of Insecticides

A pesticide, by definition, is toxic to living organism. Ideally, the toxic activity of a pesticide should be limited to its intended target organism, such as lepidopteran larvae or beetles in the case of an insecticide. However, this limitation does not always occur. Sometimes nontarget plants, insects, or other animal species may be adversely affected by the pesticide. The potential hazard to humans and wildlife is obviously a major concern following any pesticide application.

For each Insecticide Background Statement, the toxicology data from the open literature is presented in appendices and summarized in the text by taxonomic grouping (invertebrates, fish, birds, and mammals). Mammalian toxicology data is further divided into acute, subchronic, chronic, and reproductive toxicity studies. Finally, available information on mutagenicity and carcinogenicity is presented.

The following discussions provide a brief overview of toxicity testing and comparative ratings of toxicity. More complete treatment of toxicology methods can be found in Guthrie and Perry (1980), Doull et al. (1980), Loomis (1978), or Homburger et al. (1983).

2.1 Toxicity Testing

To determine the range and magnitude of potential toxic hazards associated with their use, pesticidal compounds are tested on a wide spectrum of organisms. These organisms include microorganisms, invertebrates such as insects and crustacea, fish, birds, and mammals. Tests are usually performed under controlled laboratory conditions. As such, the tests provide data on the inherent toxicity of the compounds. Although they simulate normal routes of exposure, they do not simulate natural environmental conditions. Mammals most often used are rodents, rabbits, and dogs. Farm animals such as chickens, sheep, pigs, dairy cows, and beef cattle, as well as fish, aquatic invertebrates, and wildfowl may also be used. Information from these tests regarding potential hazards can then be extrapolated to other species, including humans.

Toxicity tests in mammals fall into several categories. For all practical purposes, the duration of exposure determines whether a test is regarded as acute, subchronic, or chronic.

In an acute test conducted with terrestrial animals, a single dose or exposure is given over a short period, such as 24 hours, and the effects are observed over several days to several weeks. The most frequently determined indices of toxicity are the median lethal defined as the dose (LD_{50}) or concentration (LC_{50}) that are lethal to 50 percent of a group of treated animals. Lower numerical values for the LD_{50} or LC_{50} indicate greater toxicity than do higher values.

The acute toxicity of a compound may be based upon oral, inhalation, or dermal studies. In oral studies, the LD_{50} or LC_{50} is stated in terms of milligrams of compound administered per kilogram of body weight of the animal (mg/kg) or in terms of the parts per million (ppm) of chemical in the diet, respectively. For inhalation studies, median lethal concentrations are given in terms of parts per million for vapors and gases, or milligrams per cubic meter of air (mg/m^3) for dust or particulates. For dermal studies, test substances applied to the skin are given in terms of milligrams of compound per kilogram of body weight.

For fish and other aquatic organisms, the median lethal concentration (LC_{50}) is used as an index of acute toxicity. The LC_{50} refers to the concentration of chemical in water necessary to kill 50 percent of the aquatic test organisms during a specific exposure period. The LC_{50} is usually expressed as parts per million of chemical in water for 24, 48, 72, or 96 hours of exposure.

In addition to determining dose levels that are lethal, acute toxicity tests are also used to determine dose levels that produce eye irritation, dermal irritation and sensitization, and neurotoxic effects. Neurotoxicity testing is particularly important with organophosphate insecticides, since the nervous system is a target for these chemicals.

Subchronic studies are designed to determine adverse effects of regularly repeated doses or exposures over periods ranging from a few days to several months. Exposure may be by oral ingestion, dermal application, or inhalation. Death is usually not the endpoint. Manifestations of toxicity may include effects on the clinical health of the animal such as changes in food consumption or rate of growth, loss of weight, and behavioral changes. Signs of clinical pathology include changes in hematology, blood and serum chemistry, and urine chemistry. Animals are also examined to detect gross or histological pathologic changes. The intent is to identify target organs and the manifestations of toxicity and to define a dose level that produces a no observable effect (i.e., a NOEL or No Observable Effect Level).

Chronic studies involve the exposure of the test animals to appropriate dosages of the pesticide for a major portion of their lifespans. The objective is to assess potential health hazards that might be associated with long-term, low-level exposure. Mice and rats have been the traditional test species for chronic exposure studies because of their relatively short lifespans and the availability of extensive data on their biology in laboratory environments. Dogs are also used. Test procedures vary depending upon the toxicity endpoint being investigated.

In addition to detecting injury to various organs, tests employing sublethal levels of a particular compound are designed to detect the potential for carcinogenic, teratogenic, or adverse reproductive effects of chemicals as well as for mutagenic potential. Carcinogenicity studies are designed to determine the ability of a test substance to cause the induction and growth of malignant or benign tumors. Teratogenicity studies are conducted to detect fetal abnormalities, and reproductive studies are designed to detect adverse reproductive effects over more than one generation. Mutagenicity tests are performed to determine whether a chemical has the potential to cause an increase in the mutation rate of either (1) germ cells (eggs or sperm) or (2) somatic cells (body cells found in the various organs and tissues). Such mutations may be inherently undesirable and may also be indicative of the carcinogenic potential of the test substance.

Tests for carcinogenicity usually involve either lifetime studies or exposure of the organism to the test substance for a large portion of the organism's lifetime. In most instances, rats and mice, having relatively short lifespans, are the test organisms of choice, although dogs have been used. Statistically increased incidences of tumors (in test versus control animals) are indicative of carcinogenic potential.

Teratogenicity studies, which involve the treatment of pregnant animals, are conducted to identify agents that cause malformations (called terata) of the fetus. Although many chemicals have been shown to be teratogenic in laboratory animals, only a few compounds have been demonstrated to be teratogenic in man. Fetal malformations can also be caused by hereditary factors, as well as by such environmental factors as dietary deficiency, viral infections, hyperthermia, hormonal imbalance, and physiological stress (Loomis 1978).

Reproductive studies are performed to identify agents acting on the reproductive capacity of the animal in terms of parameters such as fertility and lactation performance. Reproductive studies also usually provide information on the effects of chemical substances on teratogenicity and, since they are often conducted through several generations, on mutagenicity. Endpoints of these tests are to determine (1) toxic effects on parents in terms of altered gonadal function, estrus cycles, mating behavior, conception rates, and early pregnancy; (2) toxic effects on fetal development in terms of normality, teratogenic/mutagenic effects, and mortality in the uterus; and (3) toxic effects on the mother in terms of lactation performance and behavior towards the offspring (Loomis 1978).

Presently, there is no unequivocal evidence for the mutagenicity of any specific chemical in humans, since mutagenicity tests are equivocal and difficult to interpret. Most mutagenicity tests are conducted on laboratory animals, microbial cells, and mammalian cells. They are designed to determine point mutations, gene mutations, chromosomal aberrations, or damage to DNA. Increases in the mutation rates of germ cells may lead to increased incidences of genetic disease in future generations; increases in the mutation rates in somatic cells may lead to increased incidences of diseases in the generation exposed to the chemical. A number of mutagenicity test systems are available and include the use of microbial or mammalian cells or whole animals and plants. Mutagenicity tests with submammalian systems, such as microorganisms (bacteria or yeast), plants, and insects, offer the advantage of simplicity and the availability of large populations to increase the probability of occurrence for rare mutation events. The disadvantage of such submammalian systems is their lack of similarity to mammalian physiology and metabolism. This disadvantage has led to the development of systems in which mammalian metabolizing systems, such as liver homogenates, are added to a bacterial test system and to the development of cytogenetic methods in which mammalian cells are examined for evidence of chromosomal changes (Loomis 1978). It should be noted that a positive result in a mutagenicity test cannot be extrapolated directly to humans, but indicates only that a chemical can act as a mutagen and as such, may pose a potential hazard to humans.

Given the assumption that a mutagenic response may be indicative of the carcinogenic potential of a substance, simple microbial test methods for the detection of mutagens can be used to screen substances for potential carcinogenic activity. More than 100 test systems exist. One of the most widely used test system is the Ames _Salmonella_/microsome _in vitro_ test, which uses mutant strains of _Salmonella typhimurium_ that require the amino acid histidine in the culture medium to grow. In the presence of mutagenic substances, these mutant strains will revert to the original, or wild type, which is not histidine dependent (Loomis 1978).

2.2 Rating of Relative Toxicities

The comparative toxicities of pesticides vary over a wide range. Several schemes have been proposed to categorize them. For example, the median lethal dose of a chemical can be given a relative toxicity rating from "highly toxic" if the LD_{50} is less than 50 mg/kg to "practically nontoxic" if the LD_{50} is greater than 5,000 mg/kg (Hodge and Sterner 1949, Radeleff 1964, Melnikov 1971). A similar system was proposed for rating subchronic toxicities in which chemicals administered in the diet are placed into five toxicity classes according to the lowest dose eliciting a toxic response (Hill et al. 1975). This system is as follows: Class I, less than 41 ppm; Class II, 41 to 100 ppm; Class III, 201 to 1,000 ppm; Class IV, 1,001 to 5,000 ppm; and Class V, greater than 5,000 ppm. The U.S. Environmental Protection Agency (EPA) guidelines for categories of acute and subchronic toxicity used for precautionary labeling under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), as amended, are shown in table 2-1. The toxicity categories range from I through IV depending upon either the median dose required to cause death or upon the severity of the reaction elicited by the chemical. All of the above ratings of relative toxicity may be referred to in the specific Insecticide Background Statements.

In terms of lethal oral acute doses in humans, a similar scheme for rating toxicity has been devised (table 2-2) and indicates the probable oral lethal dose of the different toxicity classes for an average adult human. Although such a toxicity rating system is only qualitative, it serves as a practical and useful guide by which to place toxicity categories in perspective.

Fish swimming in water containing toxicants are exposed simultaneously to the compounds by more than one route of exposure. Consequently, toxicity data for fish cannot be compared directly with toxicity data derived from studies in birds or mammals. Studies of the toxicity of pesticides to fish are conducted in laboratory environments with the fish swimming in water containing a given concentration of the pesticide formulation. These studies are either static bioassays, in which the system is not renewed during the course of the study, or flow-through bioassays, in which there is fresh test solution flowing through the exposure chambers. Data from laboratory studies must be interpreted with care when extrapolating to natural environments, since responses of fish to environmental pollutants are affected by many other environmental factors including temperature, water hardness, salinity, oxygen and carbon dioxide content, oxygen depletion, and pH (Clarke et al. 1970). In general, "cold-water" fish, such as trout and salmon, are often more sensitive to pesticides and other pollutants than the "warm-water" fish, such as carp and bass. Finally, it should be noted that laboratory aquarium studies may not always show sublethal toxic effects, such as impairment of chemical homing behavior in migratory fish, which could reduce their ability to reach spawning grounds.

Clarke et al. (1970) proposed that toxic substances with an LC_{50} for fish of less than 1 ppm for a 96-hour exposure should be considered "dangerous" (or highly toxic). Similarly, substances with an LC_{50} between 1 and 10 ppm should be considered "harmful" (toxic) and those with an LC_{50} above 10 ppm should be considered "slightly toxic." The toxicity of chemical substances to fish varies widely with substance, fish species and age, and environmental variables. Table 2-3 illustrates the range of toxicities of various substances to young rainbow trout when tested in water at 50 to 55.4 ^{O}F.

Table 2-1

Categories of Acute Toxicity

Toxicity Category	Signal Word	Oral LD_{50} (mg/kg)	Dermal LD_{50} (mg/kg)	Inhalation LC_{50} Dust or Mist (mg/liter)	Inhalation LC_{50} Gas or Vapor (ppm)	Eye Effect	Skin Irritation
I	Danger Poison	50 or less	200 or less	2 or less	200 less or	Irreversible corneal opacity at 7 days.	Severe irritation or damage at 72 hrs.
II	Warning	50 through 500	200 through 2,000	2 through 20	200 through 2,000	Corneal opacity reversible within 7 days, or irritation persisting for 7 days.	Moderate irritation at 72 hrs.
III	Caution	500 through 5,000	2,000 through 20,000	20 through 200	2,000 through 20,000	No corneal opacity, irritation reversible within 7 days.	Mild or slight irritation at 72 hrs.
IV	Caution	5,000 or greater	20,000 or greater	200 or greater	20,000 or greater	No irritation.	No irritation at 72 hrs.

Source: Adapted from U.S. Environmental Protection Agency toxicology guidelines, summarized in Ashton 1982.

Table 2-2

Toxicity Rating Chart for Acute Oral Doses in Humans

Toxicity Rating	Classification	LD_{50} (mg/kg)	Probable Lethal Oral Dose for Average Adult Human
1	Super toxic	Less than 5	A taste (less than 7 drops)
2	Extremely toxic	5 to 49	7 drops to 1 teaspoonful
3	Very toxic	50 to 499	1 teaspoonful to 1 ounce
4	Moderately toxic	500 to 4,999	1 ounce to 1 pint (1 pound)
5	Slightly toxic	5,000 to 14,999	1 pint to 1 quart
6	Practically nontoxic	15,000 and above	More than 1 quart

Source: Adapted from Heikes 1967; Hodge and Sterner 1949; Klaasen and Doull 1980; and Loomis 1978.

Table 2-3

Comparative Acute Toxicities of Selected Substances to Rainbow Trout*

Substance	Principal Use	96-Hour LC_{50} (ppm)	95% Confidence Limits (ppm)
Acephate	Insecticide	1,100	775-1,561
Fire-trol 931	Fire retardant	940	796-1,110
Ureabor	Herbicide	640	387-1,057
Glyphosate	Herbicide	130	108-156
Simazine	Herbicide	100	not given
Mirex	Insecticide	100	not given
Propham	Herbicide	38	34-43
Aminocarb	Insecticide	13.5	11.3-16.2
Diuron	Herbicide	4.9	4.1-59
Vernolate	Herbicide	4.3	3.9-4.7
Alachlor	Herbicide	2.4	1.8-3.1
Fenitrothion	Insecticide	2.4	2.0-2.9
Carbaryl	Insecticide	1.95	1.45-2.63
Coumaphos	Insecticide	0.89	0.65-1.15
Benomyl	Fungicide	0.17	0.12-0.23
DDD	Insecticide	0.07	0.057-0.087
Chlordane	Insecticide	0.042	0.037-0.048
Trifluralin	Herbicide	0.041	0.026-0.062
DDE	Insecticide	0.032	0.026-0.040
Lindane	Insecticide	0.027	0.020-0.036
Toxaphene	Insecticide	0.011	0.079-0.013
DDT	Insecticide	0.0087	0.0068-0.0114
Antimycin A	Picicide	0.000012	0.000007-0.000023

*Data from 0.8 to 1.5 g fish tested at 50 to 55.4 °F (10 to 13 °C) using technical material containing from 93 to 100% active ingredient.

Source: Johnson and Finley 1980.

3.0 Fate and Transport of Pesticides

In natural environments, pesticides are transported through various environmental compartments, stored, and/or degraded or otherwise inactivated by a variety of biological, physical, and chemical mechanisms. The fate and transport of any pesticide depends upon the physical and chemical properties of both the pesticide and the environment.

The ability of a pesticide to be transported within the environment depends primarily upon its vapor pressure, its solubility in water, and its adsorption to soil particles. Pesticides with high vapor pressures are volatile and are very mobile in the gaseous state. Unless techniques such as soil incorporation are used during application, such pesticides are readily transported through air. Highly water soluble pesticides that are not otherwise bound to soil particles can be carried into waterbodies such as rivers and streams by overland runoff following precipitation or as a result of leaching by underground water. Pesticides that are strongly adsorbed onto soil particles are not readily transported, nor are they readily available for uptake buy biological organisms in many instances.

Some pesticides tend to accumulate in animals and/or plants, while others do not. Highly toxic chemicals that bioaccumulate present a greater degree of potential harm to animals that eat the affected plants and/or prey species than chemicals that do not bioaccumulate.

Finally pesticides may be degraded in soil and water by biological, physical, and chemical processes. Pesticides are subject to biodegradation, metabolic detoxification and decomposition by organisms, or to photodegradation. Many are degraded, to varying degrees, by light.

Each of the individual Insecticide Background Statements addresses the environmental fate and transport of the subject herbicide in terms of bioaccumulation and metabolism in plants and animals, behavior in soils, and behavior in aquatic environments. Available experimental data obtained from animal bioaccumulation and metabolism studies are summarized in the text and presented in more detail in appendix F in each of the individual Insecticide Background Statements. Behavior of each herbicide in soil is discussed in terms of persistence, mobility, adsorption, chemical and microbial degradation, and volatilization. Behavior in water is similarly discussed in terms of persistence, mobility, and degradation.

3.1 Bioaccumulation and Metabolism

An understanding of the ultimate fate and transport of chemicals in the environment can only be reached with some knowledge of how readily these chemicals accumulate in living organism as well as how readily they are metabolized or eliminated from plants and animals. Such information is important for toxic substances that tend to bioaccumulate in food-chain organisms as well as for substances that are metabolized in plants or animals to toxic metabolites.

The degree to which a chemical substance is accumulated by biological systems depends upon a variety of factors, including solubility in organic solvents, solubility in water, rate of excretion by organisms, and rate of metabolism into toxic or nontoxic products. Solubility of the chemical substance in organic solvents influences its ability to pass through cell walls, mucous membranes, cuticle, and/or other surfaces of an organism and to remain in cellular or interstitial fluids. The solubility of substances in water influence the degree to which they are available for uptake by aquatic organisms. Animals accumulate toxic chemical substances by eating food containing a body burden of such substances. Accumulation of toxic substances in such organisms is influenced to a large extent by the amount of substance in the food organisms, as well as the degree to which such substances tend to be stored in tissues rather than be excreted or metabolized.

Weber (1977) compared bioaccumulation for a wide variety of pesticides, ranging from a factor in oysters of 70,000 for DDT to 0 for carbaryl (also in oysters). For comparative purposes, Weber proposed a classification of pesticides based on bioaccumulation, presented in table 3-1.

Many organisms metabolize pesticides, either into other toxic substances, nontoxic materials that are stored, nontoxic materials that are used for normal metabolic purposes, or materials that are readily excreted by the organism. These metabolic processes are generally collectively referred to as "biotransformation."

3.2 Soil

The behavior of a chemical substance in soil is determined by a number of properties relating to both the chemical and the soil environment. Properties of the chemical include water solubility, adsorption onto soil particles, chemical degradation, degradation by soil organisms, and volatility. Environmental properties include precipitation levels as well as soil type, organic matter content, temperature, and pH. Chemical substances that are highly soluble in water tend to be readily leached from soil, particularly if there are large amounts of precipitation and/or the chemical does not tend to adsorb readily to soil particles.

Adsorption of chemical substances to soil particles depends on a number of factors. Chemical substances tend to adsorb more readily to soils with higher organic matter content than to soils with low content of organic material. Adsorption rates decrease with increasing temperatures

Table 3-1

Classification of Pesticides Based on Bioaccumulation Factor

Classification	Bioaccumulation Factor*	Characteristics
Nonaccumulative	10 or less	Pesticides readily decreases when organism is removed from exposure.
Slightly accumulative	60 to 700	Pesticide is only gradually lost when organism is removed from exposure, or pesticide is degraded by the organism.
Moderately accumulative	700 to 8,000	Pesticide is only gradually lost when organism is removed from exposure, or pesticide is slowly degraded by the organism.
Highly accumulative	8,000 or more	Pesticide is not lost when organism is removed from exposure, or pesticide is not significantly degraded by the organism.

*Bioaccumulation factor is the ratio of the concentration of chemical substance in tissues of an organism to the concentration in the environment. A bioaccumulation factor of 1 indicates the same concentration in both organism and environment.

Source: Adapted from Weber 1977.

and increased soil moisture content. Soil pH has a strong influence on adsorption of chemical substances. The processes of adsorption of pesticides on soil have been reviewed in detail by Hamaker and Thompson (1972).

Chemical substances in soil are subject to degradation by chemical and biological means, as well as to movement by diffusion, dissolution, or vaporization. Chemical degradation may occur by photolysis if the substance is exposed to light at wavelengths causing its photodegradation. Many soil microorganisms are capable of metabolizing a wide variety of chemical substances and, in soils with environmental conditions fostering microbial growth, biological degradation is an important means by which substances are removed from the soil. Volatile substances are capable of moving through the soil by diffusion and of vaporizing into the atmosphere. Finally, chemical substances that are soluble in water are leached into deeper soil zones or carried by overland flow into adjacent waterbodies.

3.3 Water

The behavior of chemical substances in water is influenced by the same factors of water solubility, adsorption onto sediment, chemical and microbial degradation, and environmental conditions that influence their behavior in soils (Norris et al. 1983). Chemical substances, particularly agricultural chemicals such as pesticides, can enter aquatic systems in various ways, including direct application to surface waters, drift from spray operations close to the surface of waterbodies, mobilization of residues in ephemeral stream channels, overland flow, and leaching. The relative contribution of chemicals to waterbodies by any of these routes results from an interaction of the properties of the chemical and specific environmental factors. Generally, chemical substances that enter waterbodies are rapidly diluted, either by the large relative volumes of water in lakes and ponds, or by the addition of stream and overland flow in stream systems.

For the most part, chemical substances used in forest management applications rarely reach high concentrations in aquatic systems. The substances are rapidly diluted, are adsorbed onto sediments, or are metabolized or bioaccumulated by aquatic organisms.

4.0 Assessment of Hazards

Toxicity is an inherent property of insecticides, which are designed to kill living organisms, specifically insects. However, misuse of insecticides may also cause adverse effects on nontarget organisms, such as humans and other animals. Consequently, it is important to assess the potential hazard that could be incurred following exposure of these nontarget organisms to specific insecticides.

Proper evaluation of the hazards associated with each insecticide requires data on the toxicity of the compounds and on the level of exposure to the compounds. In practice, the toxicity of the insecticide is assessed through controlled laboratory tests, which are conducted to determine the quantity of insecticides required to produce specific toxic effects. Laboratory data can then be compared to human exposures estimated for USDA Forest Service uses to indicate if actual use practices for a particular insecticide are likely to result in adverse effects to humans or the environment.

The toxicity of noncarcinogens is assessed in terms of the no observable effect level (NOEL), while the toxicity of carcinogens is assessed in terms of potency. NOEL's are not used to assess the toxicity of carcinogens since there is evidence that there is no finite dose, or threshold, of a carcinogen that is without potential harmful effect. In the absence of a scientific consensus concerning thresholds for carcinogens, regulatory considerations adopt the conservative assumption that such thresholds do not exist.

The quantities of insecticides required to produce toxic effects are measured either in terms of the level of exposure or in terms of dose. Theoretically, dose is the actual quantity absorbed by the test animal. In actual practice with oral studies, it is generally assumed that the amount of material administered to the animal is the amount absorbed. Thus, in oral studies, the administered quantity is usually expressed as the dose. Rarely, except in metabolism studies, is the actual amount of absorption quantified.

Although the assessment of hazards is simple in principle, the necessary data are not easy to obtain, especially with regard to exposures or absorbed quantities. Actual exposure in the field is difficult to assess since application procedures vary widely, environmental and climatological conditions differ between sites, and very few studies have been conducted to determine the actual levels of exposure resulting from on-site applications of herbicides. Similarly, few studies have been conducted to determine absorbed quantities. However, based on a few exposure studies conducted during application, absorption quantities can be estimated as a percentage of the exposure level. In preparing the individual Insecticide Background Statements, a number of these estimates or assumptions have been made. The general and specific assumptions used to calculate exposures are described below in detail and are used in each Insecticide Background Statement.

4.1 General Assumptions

It is USDA Forest Service policy to use only pesticides registered by the Environmental Protection Agency. It is assumed throughout the Insecticide Background Statements that this policy is followed. Assessments of hazards associated with nonlabelled uses or application rates were not considered.

The organisms considered for hazard assessment in the Insecticide Background Statements include (at a minimum) humans, deer, rabbits, and fish. Deer represent a valuable environmental and recreational resource. Rabbits represent a worst case for exposure due to their relatively high ratio of surface area to body weight and their relatively small size, which results in a proportionately higher food intake compared to larger mammals. Fish were considered because herbicides can contaminate recreational waters. Hazard assessments for all organisms, with the exception of applicators (occupational exposure), were conducted using estimated exposure data according to the methodology described in section 4.2.

Organisms may be exposed to insecticides by dermal means (skin contact), by inhalation (breathing), and by ingestion (eating or drinking). These three routes of exposure include virtually all exposure situations. Fish may be considered to be exposed by immersion (a combination of the routes).

The hazard assessments in the individual Insecticide Background Statements consider occupational doses and environmental exposures. Specific assumptions involved in estimating occupational doses or exposures are given in the individual. Environmental exposures estimated for nonoccupational hazard assessments are used to permit comparisons with toxicological data based on known exposure levels. Environmental exposures refer to those exposures incurred directly by persons entering a forest after application operations have ceased, as well as those incurred indirectly by persons who consume animals and vegetation indigenous to the sprayed forest. Exposures incurred by representative animals (deer, rabbits, and fish) are also considered as environmental exposures for the purposes of these analyses.

Environmental exposures to humans from ingestion of contaminated material were calculated using explicitly stated assumptions for each route of exposure. No attempt is made to convert these exposure values to doses (actual absorption), since the laboratory toxicity data used for comparison are based on exposure levels rather than dose. However, in the case of oral ingestion of animal and plant material by humans, it is necessary to derive animal and plant tissue concentrations of the insecticides so that human exposure levels from such ingestion can subsequently be calculated. Calculations were based on a 50-kg person.

Occupational doses and environmental exposures are assumed to be directly related to application rate for any given insecticide. Thus, if the application rate is doubled, the occupational dose and environmental exposures are also expected to double. Within each Insecticide Background Statement, maximum reasonable occupational doses and environmental exposures are calculated. These calculations are based upon actual Forest Service application rates multiplied by occupational dose and environmental exposure factors based on a 1-lb a.e. or a.i./acre application rate. These dose and exposure factors, and the detailed assumptions involved in their derivation, are presented in section 4.2 below.

It should be noted that the calculations that follow in section 4.2 of this General Introduction, as well as the calculations in section 4 of each of the individual Insecticide Background Statements, were based on available information from various sources. The precision of measurement in the original data or estimates varied. However, to generate comparative data regarding occupational doses and environmental exposures, assumptions and mathematical operations were applied consistently for each chemical, but this resulted in indicated degrees of accuracy and precision unwarranted by the original data. For example, application data were measured to the nearest 0.1 lb a.i./acre. Estimates such as insecticide concentrations in mushrooms, on the other hand, were 1 percent of 5 ppm. This resulted in estimates at the 0.01-parts-per-million (ppm) level, which were further manipulated to estimate human exposure at the 0.001-ppm level per pound applied per acre. Although human exposure was not actually measured to the nearest ten-thousandth parts-per-million, the calculation is useful in indicating the approximate level of expected exposure. Similarly, calculated exposure levels of 0.005 mg/kg and 0.0002 mg/kg for two particular compounds may not be indicative of differences in actual measurement precision but rather may result from the mathematical operations conducted. No attempts were made to round down such values, since it was important to discern the relative differences between all means of exposure.

4.2 Specific Assumptions

4.2.1 Environmental Exposures

Environmental exposures to insecticides in humans and other animals occur through all major routes of exposure. Such exposures result from spray drift, runoff, and accumulated concentrations of insecticides in water, vegetation, and meat. Exposure decreases with time after application due to degradation and/or loss by transport or dilution of insecticide. Environmental exposures to herbicides are discussed in the Insecticide Background Statements in terms of route of exposure. Calculations were made for the organism of concern for each route of exposure, where appropriate, based on available data and/or reasonable assumptions regarding application practices and organism behavior.

Visitors to the forest, or persons downwind of the application site, may be exposed to herbicides buy dermal contact, inhalation, or ingestion. However, dermal and inhalation exposures of nonapplicators were considered to be low relative to those experienced by applicators. For example, a casual visitor to spray units would receive a dermal exposure much lower than that of workers involved in application of the herbicide. Consequently, occupational exposures to the herbicides were considered to be a maximum reasonable exposure, and direct environmental exposures to humans by dermal contact and inhalation were not considered further. However, since any human may eat vegetation, wildlife, or fish that may contain herbicide residues, direct environmental exposure from ingestion was considered. Consequently, all routes of exposure were considered for animals that may be eaten by humans.

Calculations of environmental exposures that would result from spray drift outside of the target area, such as drift into waterbodies or onto vegetation that may be eaten by humans, require consideration of spray drift. For insecticides applied aerially in a 5 mph wind, concentrations of spray drift 100 feet downwind are approximately 1 percent of those onsite (U.S. Department of the Interior 1983).

Dermal

Dermal exposure to herbicides for an animal in the spray zone is a function of the deposition rate on the animal, of the surface area contacted by the deposited spray, and of the actual amount of dislodgeable residues that actually penetrate the pelage and thus contact the skin surface. Smaller animals represent a proportionately larger surface area per unit weight. Therefore, rabbits represent game mammals with a relatively high potential exposure on a per unit weight basis compared to deer.

Actual exposures are difficult to estimate. For example, forest characteristics and animal behavior may affect deposition rate or contact area. Specifically, deposits on the forest floor were estimated at approximately one-third of the overstory canopy deposit for herbicides (Newton and Dost 1981), or 35 mg/m^2 at a 1 lb/acre application, since aerially applied sprays are partially intercepted by overstory foliage. In addition, aircraft overflights normally frighten game into seeking cover. Rabbits will likely seek their burrows. It is anticipated that deer will not run into open-area spray clouds but rather move away from spray craft or into covered areas. Since such events cannot be assumed to prevent all exposure, conservative assumptions were made.

For dermal exposures, the following assumptions were made:

- deer were estimated to weigh 80 kg and have 2.3 m^2 surface area;
- rabbits were estimated to weigh 2.0 kg and have 0.23 m^2 surface area;
- the total body surface of the animal is equally exposed to spray, either from direct deposit or contact with treated foliage as the animal walks through it; and
- 10 percent of the deposited spray actually penetrates the pelage, resulting in dermal exposure.

The estimated dermal exposure to deer is 0.101 mg/kg (35 mg/m^2 x 2.3 m^2/deer x deer/80 kg x 10 percent) for each pound of active ingredient applied per acre. The estimated dermal exposure to rabbits is 0.403 mg/kg (35 mg/m^2 x 0.23 m^2/rabbit x rabbit/2 kg x 10 percent) for each pound of active ingredient applied per acre.

Deer dermal exposure:	0.101 mg/kg	for each pound a.i. applied per care
Rabbit dermal exposure:	0.403 mg/kg	

Inhalation

Environmental inhalation exposures of animals in the spray target area would occur on a one-time basis and would be limited to a time measured in minutes. Exposure would result from inhalation of spray droplets of respirable size as well as from insecticide vapor for those substances with a high vapor pressure. Exposure by this route is expected to be of such short duration that it can be neglected.

Immersion

Exposure to fish was based on the assumptions that a 100-foot buffer zone would be maintained adjacent to streams and ponds during insecticide application and that the minimum average water depth would be 6 inches. Using the assumed buffer zone, the downwind concentration of insecticide applied in a 5-mph wind would be approximately 1 percent of the on-target concentration (U.S. Department the Interior 1983). Thus, for each pound of active ingredient applied per acre, the concentration 100 feet from the target site would be 0.1 mg/ft^2. For a waterbody with a depth of 6 inches, the resulting concentration would be 0.2 mg/ft^3, or 7.067 mg/m^3, for every pound of active ingredient applied per acre. This is equivalent to a concentration of 0.007 ppm (7.067 $mg/m^3 \times m^3$ water/10^9 mg water) for every pound of active ingredient applied per acre. To be conservative, this figure was rounded upward and exposure to fish was assumed to be 0.01 ppm per pound of active ingredient applied per acre. This estimate does not include the presence of residues that may reach waterbodies via runoff. However, the estimated concentration of 0.01 ppm for 1 lb a.i. applied per acre is higher than that of 99 percent of sample streams following actual upstream phenoxy herbicide application (Neisess 1983).

Oral

Humans and other animals may be exposed to insecticides through oral ingestion of contaminated materials. Animals may ingest plant material and water and may also be exposed because of grooming or fur licking. Humans may ingest vegetation (e.g., berries and mushrooms), game, fish, and water. Since insecticides are usually rapidly degraded or, if not degraded, translocated to often inedible plant parts, exposure by ingestion of plants in humans and other animals will be only a short-lived phenomenon. Similarly, exposure by ingestion of game, fish, and water will also be short-lived, as will animal exposure due to licking of fur.

Oral exposure of animals to aerially applied pesticides due to ingestion of water was not considered in the background statements, since estimated water contamination, as calculated, would amount to 0.01 ppm per lb a.i. applied per acre for 6-inch deep watercourses. This concentration would be less for deeper waterbodies and is several orders of magnitude less than food concentrations. Animals may consume some amounts of insecticide if they lick dewfall from foliage that has recently been sprayed; however, such exposure due to ingestion has not been adequately quantified in field studies. In addition, oral exposure due to ingestion of insecticides by licking of fur has not been quantified in field or laboratory studies.

Animal exposures due to ingestion of contaminated plant material were calculated separately for deer and rabbits due to differences in browsing patterns in these animals. Deer were assumed to browse at forest edges where foliage is maximally exposed to insecticides following aerial spraying, whereas rabbits were assumed to each foliage that is at least partially protected from deposition by overstory vegetation. Throughout the background statements, the application rate that was greatest (i.e., either aerial or ground) was used to show worst-case situations. Absent specific data, it was assumed that exposure would last 2 days.

Deer exposure calculations assume the following:

- post-spray concentration of insecticide in browse would be, as a maximum reasonable case, 100 ppm per lb of insecticide applied per acre (Newton and Dost 1981) and
- a typical deer weighs 180 lbs (80 kg) and eats 2.5 lb (1.15 kg) field weight foliage per day (Moen 1973)

Thus, daily exposure for deer due to ingestion of insecticides would be 1.44 mg/kg (100 mg/kg x 1.15 mg/deer x deer/80 kg) for each pound of active ingredient applied per acre.

Rabbit exposure calculations assume the following:

- insecticide concentrations in food foliage would be 35 ppm per lb of herbicide applied per acre (Newton and Dost 1981) and
- a typical rabbit weighs 2.0 kg, eats 60 grams of dry food daily, and receives a dose of 0.03 mg/kg/day for each ppm in food (Lehman 1959 and table 5-2 of this introduction)

Thus, rabbits would have a daily exposure due to ingestion of insecticides of 1.05 mg/kg (35 ppm x 0.03 mg/kg/day/ppm) for each pound of active ingredient applied per acre.

$$\left.\begin{array}{ll}\text{Deer or exposure:} & \text{1.44 mg/kg} \\ \text{Rabbit oral exposure:} & \text{1.05 mg/kg}\end{array}\right\} \begin{array}{l}\text{for each pound a.i.} \\ \text{applied per acre}\end{array}$$

Oral exposures to humans occur through ingestion of fish, meat, vegetation, and water. The levels of exposure would be a function of insecticide concentration in the food or water as well as the quantity of material consumed. Furthermore, not all of the ingested material would be assimilated once it is eaten by the human.

Estimates of oral exposure for human consumption of fish, which are exposed through immersion, have the following assumptions for a maximum reasonable case:

- streams and ponds containing fish will be protected from aerial spray by a 100-foot buffer zone and will be at least 6 inches deep;
- insecticide concentrations in water beyond the buffer zone are, at a maximum, 1 percent of on-target concentrations, or 0.01 ppm per pound of active ingredient applied per acre;

- a bioconcentration factor of one exists unless specific data to the contrary are available;
- insecticide residues are evenly distributed in fish tissue unless otherwise indicated by specific data;
- fish with the highest tissue concentrations are most likely to be in shallow streams and ponds and thus would be too small to be edible; and
- fish catch would be 1 kg per day for 2 days, with 50 percent of the fish biomass eaten over a 2-day interval.

Consequently, concentrations in fish tissue were estimated to be 0.01 ppm, (0.01 ppm x 1), which is equivalent to 0.01 mg/kg, for each pound a.i. applied per acre. Human consumption of 0.5 kg of fish for 2 days would result in an oral human exposure of 0.0002 mg/kg (0.01 mg/kg x 0.5 kg/day/person x 2 days x person/50 kg).

Human exposure from eating fish: 0.0002 mg/kg for each pound a.i. applied per acre

Oral exposure estimates for human consumption of both deer and rabbit meat make the following assumptions as a maximum reasonable case:

- deer and rabbit meat will come from game that may have been in the target spray zone during insecticide application and that may have eaten sprayed vegetation;
- residues of insecticide in the meat result from both oral and dermal exposure of the game, which in turn result from the level of exposure of the game to the insecticide, assimilation of the insecticide, and duration of exposure;
- assimilation by deer and rabbit is 10 percent of exposure for both dermal and oral routes unless otherwise indicated by specific data to the contrary;
- insecticide residues would be evenly distributed in meat tissue, unless otherwise indicated by specific data; and
- maximum human daily consumption of game totals 0.5 kg per day over a 2-day interval.

Deer tissue concentrations were determined by combining the maximum estimated doses resulting from dermal and oral exposures. Since dermal exposure in deer were previously determined to be 0.101 mg/kg for each pound a.i. applied per acre, the dose resulting from this exposure is estimated to be 0.01 mg/kg (0.101 mg/kg x 0.1). Since daily oral exposures were previously determined to be 1.44 mg/kg for each pound a.i. applied per acre, the dose resulting from this exposure was estimated to be 0.288 mg/kg (1.44 mg/kg/day x 2 days/ 0.1). Thus, total deer tissue concentration was the sum of the two doses (0.01 mg/kg + 0.288 mg/kg).*

Total deer tissue concentration*: 0.298 mg/kg for every pound a.i. applied per acre

Since it was assumed that a person would eat as much as 0.5 kg of deer meat per day for 2 days, the human exposure resulting from eating contaminated deer meat would be 0.006 mg/kg (0.298 mg/kg/day x 0.5 kg/day/person x person/50 kg x 2 days). It should be emphasized that not all of the ingested material would be assimilated once it is eaten by the human.

Human exposure from eating deer*: 0.006 mg/kg for each pound a.i. applied per acre

Rabbit tissue concentrations were also determined by combining the estimated doses resulting from dermal and oral exposures. Since dermal exposures were previously determined to be 0.403 mg/kg for each pound a.i. applied per acre, the dose resulting from this exposure is estimated to be 0.04 mg/kg (0.403 mg/kg x 0.1). Since daily oral exposures were determined to be 1.05 mg/kg for each pound a.i. applied per acre, the dose resulting from this exposure was estimated to be 0.21 mg/kg (1.05 mg/kg/day x 2 days x 0.1). Thus, total rabbit tissue concentration is the sum of the two doses (0.04 mg/kg + 0.21 mg/kg).*

Total rabbit tissue concentration*: 0.25 mg/kg for every pound a.i. applied per acre

Since it was assumed that a person would eat as much as 0.5 kg of rabbit meat per day for 2 days, the human exposure resulting from eating contaminated rabbit meat would be 0.0005 mg/kg (0.25 mg/kg/day x 0.5 kg/person x person/50 kg x 2 days). As with deer meat, it should be emphasized that not all of the ingested material would be assimilated once it is eaten by the human.

Human exposure from eating rabbit*: 0.005 mg/kg for each pound a.i. applied per acre

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*These calculations assume that aerial application and ground application rates are the same. When the rates differ, deer and rabbit dermal doses are based on aerial application rates and the oral doses based on the higher of the two application rates.

Oral exposure estimates for human consumption of contaminated water assume the following:

- water sources adjacent to aerial spray zones will be protected by a 100-foot buffer and will receive a maximum drift deposition that is 1 percent that of the on-target deposition;
- water sources will have a minimum average depth of 6 inches, resulting in maximum herbicide concentrations of 0.01 ppm (.01 mg/liter) at 1 lb/acre application;
- human daily consumption of water is 2 liters;
- contaminated water will be consumed for only 1 day; and
- runoff into such waterbodies from precipitation events soon after spray application may result in brief increases in concentrations of insecticide; however, due to the transitory nature of these events and the relatively small contribution of drinking water to human exposure, runoff is unlikely to be a significant contributing factor for exposure and thus need not be considered in this analysis.

Thus, the human exposure to insecticides contributed by drinking water would be 0.0004 mg/kg (0.01 mg/liter x 2 liter/person/day x 1 day x person/50 kg). As with game, it should be emphasized that not all of the ingested material would be assimilated by humans.

Human exposure from drinking water: 0.0004 mg/kg for each pound a.i. applied per acre

Oral ingestion of contaminated vegetation is considered in terms of berries and mushrooms collected at least 100 feet off of the target zone, where concentrations of herbicide on the plant material would be a maximum of 1 percent of those directly deposited on plant material within the target zone. Such an assumption assumes adequate posting of spray buffers and ignores the intentional disregard of such postings by forest visitors. The U.S. Department of the Interior (1983) has assumed a maximum of 10 ppm (10 mg/kg) pesticide on berries and 5 ppm (mg/kg) on mushrooms following aerial spray based on actual maximum measured values of phenoxy herbicides of 7 ppm in berries and 4.5 ppm in mushrooms. Specific data on spray an measurement of residues in these studies are not available. However, since reliable data on insecticide concentrations on berries and mushrooms are not available, these values are assumed in this analysis. A maximum daily consumption of 0.5 kg (for 1 day) of either berries or mushrooms was assumed. It was further assumed that collection would take place at least 100 feet off of the target zone where concentrations would be 1 percent of the on-target measurements, or 0.1 ppm (0.1 mg/kg) for berries and 0.05 ppm (0.05 mg/kg) for mushrooms. Implied are the assumptions that spray buffers will be adequately posted and that such posting will be obeyed by forest visitors. Thus, the human exposure to insecticides contributed by contaminated vegetation would be 0.001 mg/kg (0.1 mg/kg x 0.5 kg/person x person/50 kg) for berries and 0.0005 mg/kg (0.05 mg/kg x 0.5 kg/person x person/50 kg) for mushrooms. As with game and water, it should be noted that not all of the ingested material would be assimilated. In addition, much of the initially deposited material would be dislodged during picking or rinsed during washing.

Human exposure from berries:	0.0010 mg/kg	for each pound a.i. applied per acre
Human exposure from mushrooms:	0.00052 mg/kg	

4.2.2 Summary

The exposure and dose information calculated for animals and humans is summarized below, and results from an application rate of one pound a.i. applied per acre. An increase in the application rate is assumed to produce a proportionately higher number. All data are for 1 day, except for human ingestion of meat, which are for 2 days.

Environmental Exposures for Animals (per lb a.i. applied/acre)

Dermal	Rabbit = 0.404 mg/kg Deer = 0.101 mg/kg
Immersion	Fish = 0.01 ppm (water)
Oral	Rabbit = 1.05 mg/kg Deer = 1.44 mg/kg

Environmental Doses for Animals

Immersion — Fish - 0.1 ppm (tissue)

Oral and Dermal — Deer - 0.298 mg/kg*
Rabbit - 0.25 mg/kg*

Environmental Exposures for Humans (due to ingestion)

Eating fish - 0.0002 mg/kg
Eating rabbit - 0.005 mg/kg*
Eating deer - 0.006 mg/kg*
Drinking water - 0.0004 mg/kg
Eating berries - 0.0010 mg/kg
Eating mushrooms - 0.00052 mg/kg

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*These calculations assume that aerial application and ground application rates are the same. When the rates differ, deer and rabbit dermal doses are based on aerial application rates and the oral doses based on the higher of the two application rates.

5.0 Conversion Factors

A number of useful conversion factors are listed in table 5-1. Ordinarily, to obtain the desired unit, one simply multiples the known unit by conversion factor. However, this is not always the case. For example, in a feeding study to convert parts per million (ppm) of a toxicant in the diet to mg/kg/day, it is necessary to know the weight of food consumed daily and the body weight of the animal under test. Approximate relationships of ppm to mg/kg/day for a variety of experimental animals are shown in table 5-2. When the actual values for body weight of the animal and the weight of food consumed daily are known, then for 1 ppm in food the mg/kg/day is obtained by the formula:

$$\frac{\text{grams food consumed/day} \times 0.001}{\text{body weight in kg}} = \text{mg/kg/day for 1 ppm}$$

Table 5-1

Useful Conversions

Multiply	By	To Obtain
Gallon (U.S.)	3.785	Liters
Gallon (U.S.)	0.833	Gallon (Imperial)
Gallon (Imperial)	1.201	Gallon (U.S.)
Gallon (Imperial)	4.80	Quarts
Gallon	128.00	Fluid ounces
Gallon/acre	9.354	Liters/hectare
Hectares	2.471	Acres
Kilograms	2.205	Pounds
Kilograms/hectare	0.892	Pounds/acre
Kilometer	0.62	Miles
Liters/hectare	0.107	Gallons/acre
Miles	1.61	Kilometers
Ounces (fluid)	29.573	Milliliters
Ounces	28.25	Grams
Pounds	453.59	Grams
Pounds/gallon	0.12	Kilograms/liter
Pounds/acre	1.12	Kilograms/hectare

Table 5-2

Approximate Relation of Parts Per Million in Diet to mg/kg/day

Animal	Weight in Kilograms	Dry Food Consumed Per Day in Grams	1 ppm in Food Equals in mg/kg/day*	1 mg/kg/day Equals in ppm of Diet
Mouse	0.02	3	0.150	7
Chick	0.40	50	0.125	8
Young rat	0.10	10	0.100	10
Older rat	0.40	20	0.050	20
Guinea pig	0.75	30	0.040	25
Rabbit	2.0	60	0.030	33
Dog	10.0	250	0.025	40
Cat	2	100	0.050	20
Monkey	5	250	0.050	20
Dog	10	750	0.075	13
Man	60	1,500	0.025	40
Pig or Sheep	60	2,400	0.040	25
Cattle, maintenance	500	7,500	0.015	67
Cattle, fattening	500	15,000	0.030	33
Horse	500	10,000	0.020	50

$$^{*}\frac{\text{Gram food consumed/day x 0.001}}{\text{body weight in kg}} = \text{mg/kg/day for 1 ppm}$$

Source: Adapted from Lehman 1959.

6.0 Glossary

Acceptable daily intake (ADI)	The maximum dose of a substance that is anticipated to be without lifetime risk to humans when taken daily.
Acid Equivalent (a.e.)	The amount of active ingredient expressed in terms of the parent acid.
Active Ingredient (a.i.)	The agent primarily responsible for the intended pesticidal effects of a product.
Adjuvant	Substance added to a spray to act as a wetting or spreading agent, sticker, penetrant, or emulsifier in order to enhance the physical characteristics of the pesticidal materials.
Adsorb	Adherence of a substance to a surface.
Bioassay	Determination of the quantity of a substance present by the use of sensitive indicator organisms.
Carcinogen	Any cancer-producing substance.
Carrier	Material added to an active ingredient to facilitate its preparation, storage, shipment, or use.
Chronic toxicity	The poisoning effects of a series of small doses applied over a long period.
Concentration	The amount of active ingredient or pesticide equivalent in a quantity of diluent, expressed as pound/gallon (lb/gal.), milliliter/liter (ml/l), etc.
Congenital	Existing at birth but acquired in the uterus rather than inherited.
Cytological aberration	A deviation from normal cell structure or function.

Glossary (Continued)

Dose	A given quantity of test material that is taken into the body; quantity of material to be administered.
Dust	A dry, finely ground carrier material containing a pesticide active ingredient; used without further dilution.
Emulsifiable concentrate	A liquid formulation obtained by dissolving the technical active ingredient in a liquid solvent, and adding one or more emulsifiers, so that the formulated pesticide can be further diluted with water or oil for spray application.
Exposure	Application of test material to the external surfaces of a test organisms; takes into consideration route, duration, and frequency.
Formulation	(1) A pesticide preparation supplied by a manufacturer for practical use. (2) A manufacturing process by which technical active ingredients are prepared for practical use by mixing with liquid or dry diluents, grinding, or by the addition of emulsifiers, stabilizers, and other adjuvants.
Granular	A dry formulation of pesticide active ingredients mixed with or coated onto inert carrier material and other components in discrete particles generally less than 10 mm^3 in size. The carrier materials may be clays, sand, carbon, or ground corn cobs.

Hazard	The potential that the use of a pesticide would result in an adverse effect on man or the environment in a given situation.
Herbicide	A chemical used to control, suppress, or kill plants, or to severely interrupt their normal growth processes.
Intubation	oral administration of a substance by means of a tube.
LC	Lethal concentration.
LC_{50}	The median lethal concentration; the concentration of toxicant necessary to kill 50 percent of the organisms being tested. It is usually expressed in parts per million (ppm).
LC_{100}	An LC which kills all of the test organisms.
LD_{50}	The median lethal dose; the size of a single dose of a chemical necessary to kill 50 percent of the organisms in a specific test situation. It is usually expressed in the weight of the chemical per unit of body weight (mg/kg). It may be fed (oral LD_{50}), applied to the skin (dermal LD_{50}), or administered in the form of vapors (inhalation LD_{50}).
LEL	Lowest-effect level. In a series of dose levels tested, it is the lowest level at which an effect is observed in the species tested.
MLD	Minimum-lethal dose; the smallest of several doses which kills one of a group of test animals.
Mutagenic	Capable of inducing a mutation. An agent that tends to increase the occurrence or extent of mutation.

Glossary (Continued)

Mutation	A new type of organism produced as a result of a heritable change.
NOEL	The no-observable-effect-level. In a series of dose levels tested, it is the highest level at which no effect is observed, i.e., safe, in the species tested.
Nontarget vegetation	Vegetation that is not expected or not planned to be affected by the treatment.
Oncogenic (tumorigenic)	Capable of producing or inducing tumors in animals. The tumors may be either malignant (cancerous) or benign (noncancerous).
Organogenesis	The time period during embryonic development during which all major organs and organ systems are formed. During this period, the embryo is most susceptible to factors interfering with development.
Pelleted formulation	A dry formulation of pesticides and other components in discrete particles, usually larger than 10 mm^3.
Pesticide	Any substance or mixture of substances intended for controlling insects, rodents, fungi, weeds, and other forms of plants or animal life that are considered to be pests.
Photodegradation	A process of breaking down a substance through reaction to light.
Phytotoxic	Injurious or lethal to plants.
Rate	The amount of active ingredient or acid equivalent applied per unit area or other treatment unit.

Glossary (Continued)

Residue	That quantity of pesticide, its degradation products, and/or its metabolites remaining on or in the soil, plant parts, animal tissues, whole organisms, and surfaces.
Risk	The probability that a substance will produce harm under specified conditions.
Safety	The reciprocal of risk, i.e., the probability that harm will not occur under specified conditions.
Selective pesticide	A chemical that is more toxic to some species than to others.
Soluble powder	Similar to a wettable powder, except that the technical active ingredient as well as the other formulation components will dissolve in water or other liquid for which the powder is formulated.
STEL	Short-term exposure limit. The maximal allowable concentration, or ceiling, not to be exceeded at any time during a 15-minute exposure period.
Surfactant	A material that favors or improves the emulsifying, dispersing, spreading, wetting, or other surface modifying properties of liquids.
Susceptibility	Capacity to be adversely affected by pesticide treatment.
Technical active ingredient	The active chemical along with unavoidable impurities remaining from the manufacturing process.
Teratogen	Any substance capable of producing structural abnormalities of prenatal origin, present at birth or manifested shortly thereafter (the ability to produce birth defects).

Glossary (Continued)

Threshold	A dose or exposure below which there is no apparent or measurable adverse effect.
TL_m (TL_{50})	Median tolerance limit; the concentration of chemical in water necessary to kill 50 percent of the test aquatic organisms during a specific exposure period. The TL_m is usually expressed as parts per million parts of water for 24, 48, 72, or 96 hours of exposure.
TLV-TWA	Threshold limit value-time weighted average. The time-weighted average concentration for a normal 8-hour workday and a 40-hour work week, to which nearly all workers may be repeatedly exposed without adverse effect.
Tolerance	(1) Capacity to withstand pesticide treatment without adverse effect on normal growth and function. (2) The maximum residue concentration legally allowed for a specific pesticide, its metabolites, or breakdown products, in or on a particular raw agricultural product, processed food, or feed item. Expressed as parts per million (ppm).
Toxicity	(1) The capacity or property of a substance to cause any adverse effects. It is based on scientifically verifiable data from animal or human exposure tests. (2) The specific quantity of a substance that may be expected, under specific conditions, to do damage to a specific living organism.

Glossary (Concluded)

Translocation	Movement of a pesticide or other substance within a plant via the phloem or xylem.
TWA	The time-weighted average concentration is the average exposure concentration based on the duration of exposure to airborne concentration as it varies during an 8-hour workday.
Wetting agent	A substance that serves to reduce interfacial tensions and causes spray solutions or suspensions to make better contact with treated surfaces.
Wettable powder	A finely ground, dry powder formulation that can be readily suspended in water for application in spray equipment.

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Acephate Summary

Major Trade Names: Orthene®, Orthene 75 SP, Orthene Forest Spray, Ortho 12420®, Ortran®, Chevron RE-12420®, RE-12420®, ENT 27822®, Orthan®, Orthene 2.6EC®, Orthene 10G®, Orthene 80 See Protector®.

Chemical Names: O,S-Dimethyl acetylphosphoramidothiate.

Major Forestry Applications: Western spruce budworm, spruce budworm, gypsy moth, needleminers in forests and grasshoppers in rangeland.

Toxicology

Acephate is 50 times less toxic to mammals than its main metabolite, methamidophos. Acephate has a slight acute toxicity by oral exposure and may cause eye or skin irritation from repeated contact. It has a slight acute toxicity by inhalation. No carcinogenic activity is known; however, some teratogenic and mutagenic effects have been shown.

In birds, sublethal effects may occur as a result of inhibition of cholinesterase activity in the brain. A 50 percent reduction can be lethal. Normal rates used in the field do not cause mortality in birds, wild mammals, amphibians, or fish. Nontarget honey bees and other pollinators are affected by acephate. Aquatic invertebrates take up acephate from water and sediments and convert it to the more toxic methamidophos. No mortality was observed and levels were decreased within hours following the release of acephate. No chronic effects were reported.

Acephate acts as a contact poison on target organisms and penetrates the outer surface. In plants, acephate acts as a systemic poison and is carried to leaf surfaces where it then contacts the pest or is ingested. Like other organophosphorus pesticides, acephate acts as a nerve poison on the pest and intoxicates by acetylcholinesterase inhibition.

Environmental Fate

Acephate is only moderately persistent in the environment and is biodegraded in soils. Microbial degradation is the mechanism for decomposition in soils. It has a reported half-life in soils from 1.5 to 13 days depending on soil characteristics and environmental conditions. Acephate has a relatively high soil mobility. It is readily moved by soil water with little retention on soil particles. Persistence in water is pH dependent, with half-lives ranging from 19 to 55 days over a pH range of 9 to 5, respectively. Persistence on leaf surfaces exceeds persistence in water or soil. Plants acquire acephate from treated soil and move it to their leaf surfaces, where it accumulates. Residues remain at high levels for about 3 weeks. Animals metabolize acephate and excrete it rapidly. It appears to have relatively low, if any, bioaccumulation in birds and mammals. When used at recommended application rates, current evidence indicates that acephate poses a small risk to humans via the food chain.

Exposure and Hazard

No chronic or carcinogenic evidence exists for acephate, but some teratogenic and mutagenic risks are possible. Major exposure pathways are: oral, by eating contaminated plants or fish or by drinking contaminated water; dermal, by occupational exposure or from being in a spray zone; or inhalation, by occupational exposure or from being in a spray zone. Acephate also is known to drift out of spray zones. Exposure studies are lacking to assess risk to the public and workers. Epidemiology studies are lacking for cancer, birth defects, and systemic effects. It is possible that realistic doses over a long time frame could cause temporary depression of cholinesterase activity. Worst-case doses (e.g., occupational or eating contaminated foods) would probably cause some systemic effects and a measurable cholinesterase inhibition. Fatalities are unlikely from short-term contact. Accidents resulting in spills could cause dermal exposure of contaminate drinking water. Some accident scenarios could result in human health effects.

1.0 General Information

Acephate is an organophosphate contact and systemic insecticide used against a wide variety of terrestrial insect pest species in crops, forests, and grasslands. The U.S. Department of Agriculture (USDA), Forest Service, typically uses acephate to control gypsy moth larvae, western spruce budworm, and spruce budworm on forests and grasshoppers on rangelands of the West. Trade names include Orthene®, Ortho 12420, Orthene Forest Spray, Ortran, and Ortho 9006; the latter is methamidophos, a metabolite of acephate. Synonyms and trade names for acephate are as follows (Larson 1983):

- Chevron RE-12420
- ENT 27822
- Orthan
- Orthen (German)
- Orthene Concentrate
- Orthene 2.6EC
- Orthene 10G
- Orthene 75 SP
- Orthene 80 Seed Protectorate
- Orthene EPN

All of the Orthene products are manufactured and sold by Chevron Chemical Company, Ortho Division. Commercial production began in 1972 and registration was granted for gypsy moth control in 1976 (Lambert 1983).

The chemical name for acephate is O,S-dimethyl acetyphosphoramidothiate (CAS number 30560-19-1). It is a stable solid-state chemical, soluble in water, moderately soluble in alcohol and acetone, slightly to moderately soluble in aromatic solvents. As a white powder, acephate has a strong mercaptan-type odor. Acephate's bulk density is 0.4 to 0.6 g/cm^3 It is relatively stable to hydrolysis, sensitive to heat, and slightly hygroscopic. It is moderately persistent with residual systemic activity of approximately 10 to 15 days at normal application rates on foliage. Residues may persist for a year after application.

1.1 Normal Use Patterns

The standard formulation of acephate is as a soluble powder. However, a number of application methods have been tested for soluble powder including water-based sprays, acetone solutions, encapsulated slow-release pellets, granular formulations, and implant devices (Lambert 1983). Normal patterns include: household sprays for ornamental plants; greenhouse sprays for ornamentals and selected vegetables; backpack, vehicular, and aerial sprays for crops; and aerial sprays for forested areas.

Orthene Forest Spray [75 percent active ingredient (a.i.) acephate] is used for foliage protection against gypsy moth larvae in infested forests by aerial application of 0.66 to 1.0 lb/0.5 gal water/acre (0.80 to 1.22 kg/ha). This information is found on the labels of Orthene products. Helicopter application is recommended for suppression of larval populations of western spruce budworm at rates of 0.66 lb/gal water/acre (0.80 kg/ha). Aerial application is recommended by the manufacturer for suppression of larval populations of spruce budworm at concentrations of 0.66 lb/0.5 gal water/acre (0.80 kg/ha) or 0.5 lb a.i./0.5 gal.

Orthene 75 SP (Soluble Powder) 75 percent a.i. is used to control range grasshoppers and a number of crop pests. Low rates 0.125 to 0.166 lb/acre (0.094 to 0.125 lb a.i./acre) (0.15 to 0.20 kg/ha) are used for pasture and rangeland grasshopper control. Wooldridge (1983) reported that acephate applied aerially at a rate of 105 g a.i./ha mixed with an antidrift additive provided excellent control of 14 species of grasshoppers on rangeland 10 days after treatment.

On crops, the Orthene 75 SP rates recommended on the label vary from 0.33 lb/acre (0.40 kg/ha) to 1.33 lb/acre (1.62 kg/ha). This insecticide is used on beans, lima beans, cranberry, celery, cotton, lettuce, peanuts, peppermint, spearmint, soybeans, and sweetpeppers. Crop pests controlled by acephate include the following: grasshoppers, fleahoppers, leafhoppers, aphids, bean leaf beetles, bean leafrollers, cabbage loopers, cutworms, green cloverworms, Mexican bean bettles, oak moths, plantbugs (Lygus), soybean loopers, thrips, webworms, whiteflies, beet and fall armyworms, corn earworms, European corn borers, green peach aphids, bollworms, tobacco budworms, velvet bean caterpillars, alfalfa loopers, stinkbugs, threecornered alfalfa hoppers, and tobacco hornworms. Acephate can be used to within 21 days before crop harvest, hay use, or grazing animals on treated pastures. Acephate is also used for spot treatment of cockroaches.

The USDA Forest Service uses acephate for spruce budworm in the West and gypsy moth larvae in the East. The latter use is minimal, from 1 to 400 acres/year (table 1-1). Rates recommended by the manufacturer are 0.8 to 1.22 kg/ha for gypsy moth control. For western spruce budworm, the USDA Forest Service uses Orthene Medicaps annually to protect grand and Douglas-fir as single systemic implants of cartridges (0.8 a.i.) (Reardon 1984). Lodgepole needleminers are controlled by acephate as spray and well as via trunk implant (Brown et al. 1978). Spray programs for acephate are limited.

1.2 Physical and Chemical Properties

The active ingredient of acephate is O,S-dimethyl acetylphosphoramidothiate, which has the following empirical formula:

$C_4H_{10}NO_3PS$ (molecular weight 183.16)

Table 1-1

USDA Forest Service Acephate Use

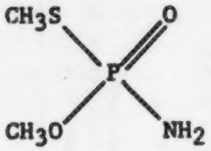
Purpose	Active Ingredient (ave. lb a.i./acre)	Acres Treated	Formulation	Application Method
1979				
Gypsy moth	0.75	400	Acephate Liquid Solution	Aerial (2)
1981				
Gypsy moth	0.66 to 1.0	20	Acephate Liquid Solution	
Western Spruce Budworm	0.5			
Spruce Budworm	0.5			
1982				
Gypsy moth	0.66 to 1.0	120	Acephate Liquid Solution	Aerial (2)
Western Spruce Budworm	0.5			
Spruce Budworm	0.5			
1984				
Gypsy moth	0.66 to 1.0	<1	Orthene Forest Spray Solution powder	Backpack Spray
Western Spruce Budworm	0.5			
Spruce Budworm	0.5			

Technical Orthene is a white hygroscopic solid with a melting point of 92 to 93 °C (pure). It is extremely soluble in water, methylene chloride, and ethanol and quite soluble in acetone, and less soluble in ethyl acetate, benzene, toluene, and ether in descending order (Leary 1973). Hydrolysis of acephate is quantitatively the same in acid, base, and neutral solution. In each case, P-N cleavage takes place to give dimethyl hydrogen phosphorothiate. This chemical is relatively stable in acid solution, but degrades more rapidly in base. The N-C bond does not cleave in the biological pH range (5 to 8). There is evidence of a small amount of hydrolysis of this bond at extreme acid and base pH values (Leary 1973).

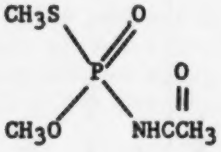
Acephate is incompatible with alkaline materials, including hypochlorite oxidants and may produce noxious gases. Normal combustion forms carbon dioxide and water vapor and may produce oxides of sulfur, nitrogen, and phosphorus; incomplete combustion can produce carbon monoxide.

Magee (1974) described the detailed structure and bioactivity of Orthene insecticide analogs, including Monitor®. Analogs with variations of O, S, and N substitution were prepared and tested; with few exceptions, the best analogs of Orthene were found to combine high insecticidal activity with moderate to low mammalian toxicity. Several years later, Umetsu et al. (1977) isolated seven impurities in acephate and reported reduced mammalian toxicity after storage of technical acephate for 3 to 6 months at 40 °C. The metabolite that inhibits cholinesterase (ChE) has now been isolated as methamidophos that is also required by U.S. EPA (1983b).

Orthene is the acetyl derivative of methamidophos, trade name Monitor. Both have approximately the same insecticidal acute toxicity, but Orthene is safer for mammals. Acephate is metabolized by insects into methamidophos that is the actual lethal agent. In mammals, relatively little methamidophos is formed (Wustner et al. 1978, in Lambert 1983). The chemical structures of Orthene and Monitor are compared below:



MONITOR



ORTHENE

2.0 Toxicity of Acephate

Acephate is expected to have slight acute toxicity by ingestion, and the (U.S. EPA) has set tolerance limits for raw agricultural commodities as low as 0.5 ppm for cranberries (U.S. EPA 1983a) ranging up to 15 ppm (U.S. EPA 1983a) on grass and hay. A food additive tolerance of 0.02 ppm was established for combined residues of acephate and its ChE-inhibiting metabolite methamidophos (U.S. EPA 1983b) for both products. Acute and subchronic toxicity properties are summarized in table 2-1.

Acephate may cause eye irritation or skin irritation from prolonged or frequent repeated contact. It is expected to have a slight acute toxicity by inhalation. The inhalation LC_{50} (rat) for a 1-hour exposure was greater than 11 mg/l (Chevron 1984). Acephate did not display any mutagenic activity (U.S. EPA 1983a). Few data exist on carcinogenicity. Chevron (1984) states that Orthene has not been associated with cancer or chronic toxicity. Some teratogenic effects have been observed, and the no observed effect level (NOEL) for rabbits is 10 mg/kg and for rats is 200 mg/kg.

2.1 Microorganisms

Acephate has been shown to be mutagenic to some species of microorganisms (Salmonella typhimurium, Escherichia coli, and Saccharomyces cerevisiae). Toxicity data are summarized for microorganisms in appendix A and table 2-1.

Williams and Albright (1983) summarized the effects of acephate on aquatic bacteria from a Canadian lake. Six bacterial isolates (gram-negative rods) were tested in a growth medium containing 1,000 ppm acephate. Only Chromobacterium lividum showed reduced growth. No change was observed in other bacteria doubling times. Acephate increased doubling time, decreased maximum cellular yield, and reduced cell size of Chromobacterium lividum in high concentrations (1,000 ppm). Total [^{14}C] glucose and ^{14}C-labeled amino acid uptake rates were reduced by the high acephate concentrations. The high concentration did not affect active uptake of cycloleucine, a nonmetabolized amino acid analogue. Low concentrations (1 ppm) of acephate had little influence on metabolic processes. The authors suggest that the high acephate concentration affects biosynthetic mechanisms rather than directly affecting active transport mechanisms.

In a soil microorganism study, Focht and Joseph (1974) treated soils with acephate (20 ppm) (70 percent technical) and another with 20 ppm Monitor. No adverse effects were reported after 50 days. Population levels of actinomycetes, bacteria, and fungi were not substantially affected. Neither could a marked effect be shown for ammonification,

Table 2-1

Summary of Toxicological Properties of Acephate

1. Acute Oral Toxicity

Organism (Formulation)	Exposure	Adverse Effects
Rat (acephate)	Not specified.	LD_{50} = 700 mg/kg
Rat (acephate; technical)	Not specified.	LD_{50} = 945 mg/kg for males; LD_{50} = 866 mg/kg for females.
Rat (acephate)	Not specified.	No signs of neurotoxicity at 375 mg/kg.
Rat (acephate; technical)	Not specified.	LD_{50} = 1,800 mg/kg
Rat (acephate; purified)	Not specified.	LD_{50} = 1,480 mg/kg
Mouse (acephate; technical)	Not specified.	LD_{50} = 500 mg/kg
Mouse (acephate; purified)	Not specified.	LD_{50} = 425 mg/kg
Mouse (acephate; 5% impurities)	Not specified.	LD_{50} = 520 mg/kg
Mouse (acephate plus specific impurities)	Not specified.	LD_{50} = 570 mg/kg for acephate; LD_{50} = 720 mg/kg, 540 mg/kg, and 460 mg/kg for acephate plus 1% O,O-dimethyl-N-acetylphosphoramidthioate and acephate plus 0.5% and 1%, O,O,S-trimethylphosporodithioate, respectively; LD_{50} = 570 mg/kg for acephate plus 5% methamidophos.
Mouse (acephate; technical)	Not specified.	LD_{50} = 500 mg/kg for unstored acephate; LD_{50} = 640 mg/kg for acephate stored 3 mos at 20 °C; LD_{50} = 720 mg/kg for acephate stored for 6 mos at 20 °C; LD_{50} = 790 and 820 mg/kg for acephate stored at 40 °C for 3 and 6 mos, respectively.

Table 2-1 (Continued)

2. Acute Dermal Toxicity

Organism (Formulation)	Exposure	Adverse Effects
Mouse (acephate)	Not specified.	LD_{50} = 233 mg/kg
Dog (acephate)	Not specified.	LD_{50} = 681 mg/kg
Rabbit (acephate)	Not specified.	LD_{50} = 2,000 mg/kg
Rabbit (acephate)	Not specified	LD_{50} = 10,250 mg/kg

3. Subchronic Toxicity

Organism (Formulation)	Exposure	Adverse Effects
Rabbit (acephate)	Given 5 ppm and other unspecified doses in food for 90 days.	NOEL = 0.25 mg/kg based on inhibition of ChE activity in plasma, red blood cells, and brain tissue.
Rat (acephate 75 SP)	Given oral doses of 60 mg/kg/day (diluted in water) for 2, 4, 6, or 8 wks. Deprived of food 18 hrs prior to sacrifice.	Slight increases in total lipid at 4, 6, and 8 wks. Small, but statistically significant decreases in serum total lipids and triglycerides. No other systemic toxicity effects reported.
Rat (acephate 75 SP)	Given oral doses of 60 mg/rat/day for 30, 45, or 60 days rats weighed 100 to 950 g each.	Succinic dehydrogenase activity and levels of glutathione in blood and liver were decreased in 60-day treatment group. Lactic dehydrogenase activity in 60-day treatment group was elevated. No systemic toxicity effects reported.

Table 2-1 (Continued)

4. Chronic Toxicity

Organism (Formulation)	Exposure	Adverse Effects
Rat (acephate)	Given 5 ppm and other unspecified doses in food for 28 mos.	NOEL = 0.25 mg/kg based on inhibition of ChE activity in plasma, red blood cells, and brain tissue.
Dog (acephate)	Given 30 ppm, 100 ppm, and other unspecified doses in food for 2 yrs.	NOEL = 0.75 mg/kg based on inhibition of ChE activity in plasma, red blood cells, and brain tissue. NOEL = 2.5 mg/kg based on systemic toxicity.
Humans (acephate)	Occupational exposure; not specified.	No adverse health effects and no inhibition of blood ChE activity. Acephate detected in urine at levels up to 5 ppm.

5. Reproductive and Teratogenic Effects

Organism (Formulation)	Exposure	Adverse Effects
Rat (acephate)	Highest dose tested was 200 mg/kg. No additional details available.	NOEL = 200 mg/kg for teratogenic effects.
Rabbit (acephate)	Highest dose tested was 10 mg/kg. No additional details available.	NOEL = 10 mg/kg for teratogenic effects.

Table 2-1 (Continued)

6. Mutagenicity

Organism	Formulation	Adverse Effects
Salmonella typhimurium (TA100)	Acephate	Mutagenic; 0.0013 revertants per nano-mole.
S. typhimurium (TA1535, TA1537, TA1538 TA100)	Acephate	Weakly mutagenic in TA100.
S. typhimurium (TA1535, TA1537, TA1538, TA98, TA100)	Acephate	Mutagenic in TA100 with and without S9 activation.
S. typhimurium (hisC117, hisG46, TA1530, TA1535)	Acephate (Orthene 75)	Not mutagenic.
S. typhimurium (hisG46)	Acephate (nitrosated with sodium nitrate)	Not mutagenic.
S. typhimurium (SL4525 and SL4700)	Acephate	Not mutagenic.
Escherichia coli (WP2, WP2uvrA, CM561 CM571, CM611, WP67, WP12)	Acephate (Orthene 75)	Mutagenic in strains WP2, WP2uvrA, WP67, and WP12 without S9 metabolic activation.
E. coli (WP2 hcr)	Acephate	Mutagenic.
E. coli (WP2)	Acephate	Not mutagenic.
E. coli (W3110 and P3478)	Acephate	Inconclusive.
Bacillus subtilis (H17 and M45)	Acephate	Inconclusive.

Table 2-1 (Concluded)

6. Mutagenicity (Concluded)

Organism	Formulation	Adverse Effects
Saccharomyces cerevisiae (D3 and D7)	Acephate	Mutagenic in D3 and D7.
S. cerevisiae (D3)	Acephate	Mugatenic.
S. cerevisiae (D7)	Acephate	Mutagenic with S9 metabolic activation.
S. cerevisiae (D7)	Acephate	Mugatenic.
Human fetal lung fibroblasts (WI38)	Acephate	Mutagenic without S9 metabolic activation. Increased unscheduled DNA synthesis.
Mouse lymphoma L5178Y cells	Acephate	Mutagenic.
Chinese hamster ovary cells	Acephate	Mutagenic; induced sister chromatid exchanges above control incidence level.
Mouse bone marrow and cardiac blood cells	Acephate	Not mutagenic; did not induce chromosome breakage (micronuclei).
Drosophila melanogaster	Acephate	Not mutagenic in sex-linked recessive lethal assay at 10 ppm in food for 48 hrs.
D. melanogaster	Acephate	Not mutagenic in sex-linked recessive lethal assay.

nitrification, sulfur oxidation, or respiration rates. It was concluded by these authors that acephate, even at levels 10 times greater than normally used in the field, had no major effect on soil microflora.

2.2 Invertebrates

Acephate is highly toxic to a wide variety of insects, including nontarget honey bees. Toxicity studies are summarized in appendix B for invertebrates. Effects on target species are summarized in table 2-2.

The following discussions are based first on the effects of acephate in a variety of formulations on target terrestrial insect species, followed by a summary of literature on effects on nontarget invertebrates.

Leigh et al. (1977) were concerned with growing resistance of plantbugs (Lygus hesperus) to organophosphorus and carbamate insecticides used on crops with a greater history of insecticides use. LD_{50}'s (median lethal doses) were measured from 1968 to 1975 for 19 insecticides. The LD_{50} of acephate ranged from 0.131 µg/specimen in 1971 to 0.158 µg/specimen in 1972, but no apparent resistance developed.

Rogers and Howell (1973) measured the toxicity of acephate to harlequin bugs (Hemiptera, Pentatomidae) on cabbage. Treatment was in 40 gal. of water/acre. Acephate was significantly more effective ($p = 0.05$) in reducing adult populations than diazinon. Acephate reduced adults by more than 99 percent. Acephate at 0.75 and 2.0 lb a.i. significantly reduced egg populations by the third day. By day 7, only the 2 lb a.i. treatment was effective for eggs.

Wolfenbarger and Harding (1980) compared several alkyl and aryl phosphormaides insecticides against Heliothis spp. (Lepidophtera, Noctuidae). The research test included acephate applications in the field and laboratory to the tobacco budworm (Heliothis virescens) from 1972 to 1976. Acephate applied topically (30 to 50 larvae in 2 to 3 replicates) was toxic with an LD_{50} value of 0.041 mg/g for 3rd-stage larvae. In field tests, acephate sprayed at rates of 1.12 kg/ha in cotton was effective in controlling the tobacco budworm damage, i.e., there was a significant reduction in boll damage.

Foster et al. (1983) tested acephate on 17 species of grasshoppers on rangelands at rates ranging from 105 to 140 g a.i./ha in volumes of 2,337, 1,899, and 1,461 ml of mix/ha with the addition of a drift retardant. The Orthene 75 SP plots had an 80 to 94 percent mortality in 4 days, and 87 to 97 percent mortality in 10 days. For application volumes of 2,337 ml/ha at 10 days, there was no significant difference between the 140 or 105 g a.i./ha with or without the drift retardant. Orthene concentrate

Table 2-2

Effects of Acephate on Target Organisms

Organism	Formulation	Effect	Reference
Lepidoptera Tobacco budworm (*Heliothis virescens*)	Acephate	Significant reduction of boll damage	Wolfenbarger and Harding 1980
Potato tuberworm (*Phthorimaca*) *operculella*)	Orthene	LD_{50} = 126 to 1,770 μg	Lambert 1983
Spruce budworm (*Choristoneura occidentalis*)	Orthene medicaps	Significant reduction in larvae LC_{50} = 14 to 18 ppm	Reardon and Haskett 1981, Reardon 1984,
	Orthene	LD_{50} = 21.6 to 55.7 μg/g	Lambert 1983
Nettle caterpillars (*Setora nitens*) (*Thosea asigna*)	Orthene 75 SP	Unsuccessful control at 6 a.i./tree	Hutauruk and Sipayung 1978
Cutworm larvae (*Peridroma savcia*)	Acephate (<0.1 to 0.5%)	60 to 100% mortality depending upon species of plant	Berry et al. 1980
Orthoptera 14 species grasshopper	Acephate 140 + 105	87 to 97% mortality after 10 days	Foster et al. 1983
Hemiptera Harlequin bug (*Murgantia histrionica*)	Acephate (5%)	99.8 to 100% adult mortality (1 to 2 lb/acre)	Rogers and Howell 1973
(*Lygus hesperus*)	Acephate	24 hr LD_{50} = 0.13 to 4.16 μg/specimen	Leigh et al. 1977
Pine needleminer (*Coleotechnites milleri*)	Acephate 75 SP + medicaps	Solution 94.1% effective; medicap 49% effective	Brown et al. 1979
Leafminer (*Liromyza sativae*)	Acephate 75 SP	84 to 94% effective	Lindquist and Kueger 1975

Table 2-2 (Concluded)

Organism	Formulation	Effect	Reference
Diptera (*Chironomous*)	94% technical Acephate	LC_{50} = >1000 mg/l	Johnson and Finley 1980
Housefly (*Musca domesticus*)	Acephate	ChE inhibition lethal in 12 hrs	Rojakovick and March 1972
Blackfly (*Aleucocanthus woglumi*)	Acephate and Methamidophos	Residue persisted 3 mos	Fitzpatrick and Bogan 1980
Coleoptera Red flour beetle (*Tribolium castaneum*)	Acephate	95% mortality; 0.55 to 0.89 mg/g topical	Zetter and Jones 1977
Myzus persicae	Acephate	500 ppm <1% living on 4th day	Mowlam et al. 1977
Homoptera Green rice leafhopper (*Nephotottix cincticeps*)	Orthene	LD_{50} = 7.1 to 12.6 ppm	Lambert 1983
Neuroptera Green lacewing (*Chrysopa carnea*)	Orthene	LD_{50} = 106 μg/g	Lambert 1983

site mortality was from 88 to 100 percent, and Orthene 75 SP mortality was from 97 to 100 percent.

Orthene is also used in Medicaps formulation for western spruce budworm control by systemic injection on both deciduous and coniferous trees. The insecticide, 85 percent acephate, was inserted via cartridge into a hole drilled into the tree. Reardon and Haskett (1981) and Reardon (1984) reported the Medicaps as effective on western spruce budworms (Choristoneura occidentatis) on grand and Douglas-fir. Dosage of one cartridge (0.8 g a.i./10 cm) per tree applied three times reduced larvae to less than 10/100 buds. Acephate and methamidophos residue levels in foliage were sufficient to cause larval mortality 28 days after treatment. Reardon (1984) reported the residues present 1 year after treatment. For Douglas-fir, two annual treatments reduced populations by 62 percent.

Trunk implants of Medicaps also are reported by Brown et al. (1979) as effective against lodgepole needleminers (Coleotechnites milleri) in three experiments in 3 years, with acephate residues measured in the latter 2 years.

Both acephate spray (3.4 l/tree) and trunk implants (1.5 g dry insecticide = 1.25 g a.i.) were effective against larvae. The acephate solution injected was 1 ml (= 0.18 g a.i.) made of 120 g acephate 75 SP dissolved in 500 ml water containing 1 ml of buffer. About 4 weeks later, acephate mortalities were determined. The mortality of larvae ranged from 56.1 to 99.2 percent. Residues remaining were 65 to 222 ppm, respectively. Acephate spray residues degraded rapidly initially, whereas solution implant residues increased rapidly, and implants residue levels increased slowly over 4 weeks.

Zettler and Jones (1977) compared the toxicity of seven insecticides to malathion-resistant red flour beetles. No cross resistance was observed for acephate at the LD_{50} level 0.138 mg/g of insect body weight, but low levels of cross resistance were observed at the LD_{95} level (0.551 mg/g). Acephate was not as toxic to the malathion-resistance beetles as pirmiphosmethyl, dichlorvos, or naled.

Acephate action in the soil is a concern following spray programs because of potential impacts on soil organisms. Lindquist and Wolgamott (1980) tested toxicity on soil mites and a predator (Tetranychus urtical and Phytoseiulu persimilis). Acephate was both directly toxic and toxic via the food chain to the predator. Acephate applied as soil drenches at rates of 75, 150, and 300 ppm was more toxic by this route than foliar sprays, in some cases killing all the predators within 21 days. Residual and food chain effects could not be separated. Direct contact appeared to have low toxicity. Greatest mortality was exhibited when acephate was applied as a soil drench.

Acephate is highly toxic to nontarget honey bees. Laboratory and controlled field application tests conducted by Kupetz et al. (1979 in Lambert 1983), indicated the following LD_{50} values for acephate:

Oral (feeding studies) = 1.07 µg/g body weight
Topical (direct application) = 5.4 µg/g body weight
Contact (with deposit on surfaces) = 3,000 µg/100 cm^2

Acephate was tested as a candidate control chemical for the Douglas-fir tussock moth as reported by Robinson and Johansen (1978, in Lambert 1983). Part of the test was to determine the effects of acephate and honey bees and other pollinators in the forest environment. Honey bee colonies in the path of aerial spray applications were all technically dead within 45 to 48 days after treatment, and colonies in untreated plots near the treated areas were also adversely affected. Data from this study indicated that foraging workers succumbed to the acephate in the field. Application rates were 1 and 2 lb a.i./acre on selected plots in the Wallowa-Whitman National Forest located in northeastern Oregon (Davis et al. 1978, in Lambert 1983).

Moffett et al. (1979) reported results of four mitigation measures to reduce losses of honey bees (Apis mellifera). Measures tested were covering with burlap, pollen feeding, providing shade, and using a Wardecker waterer. Eighteen treatments, two with acephate, were tested on 180 colonies (10 replications/treatment). Fifteen acephate-treated fields were sprayed with 748 g/ha (Orthene) or 1.121 g + 2.34 liters/ha (Orthene and Paratox) in September at volumes of 28.06 liters/ha. Overall both covering and pollen feeding were the most effective. Although all colonies suffered severe losses from the spraying between 80 to 100 percent of the protected colonies survived compared to 20 percent survival in controls or ineffective treatments. Most of the colonies survived and lived through winter (96 of 102); queens laid well and did not appear to be permanently damaged.

Acephate applied to three experimental plots in a northeastern Oregon forest at 1 and 2 lb a.i./acre had a devastating effect on ants of the genus Formica (Roush and Akre 1978, in Lambert 1983). The insecticide was applied during relatively cool weather. As the temperature rose, foraging activities of the ants increased, and foraging material containing acephate was brought to the colonies. The colonies were effectively dead 6 days after spraying and did not recover during the following year. Yellow jackets observed during the same study showed no apparent effects due to acephate applications.

Acephate is slightly to moderately toxic to nontarget aquatic invertebrates within the spray area. The U.S. Fish and Wildlife Service toxicity tests (Johnson and Finley 1980) determined the 96-hour LC_{50} doses to four species of aquatic insects important to fish food chains.

Laboratory tests were conducted using 94 percent technical and 74 percent soluble powder acephate. Temperatures ranged from 7° to 20 °C, pH from 6.5 to 9.5, and hardness from 12 to 300 ppm. The least tolerant species was the stonefly naid (Pteronarcella). The LC_{50} for this species was 9.5 (7.3 to 12.3) mg/l, in the moderately toxic range. Increasing the pH from 6.5 to 8.5 increased the LC_{50} for stoneflies from 6.4 to 21 mg/l. Acephate was slightly toxic for the Skwala and Gammarus. The LC_{50} for Gammarus was higher (>50 mg/l) than the LC_{50} for Skwala, which was 12 mg/l for both the 94 percent technical and 74 percent soluble powder form of acephate. The larvae of the midge (Chironomus) was the most tolerant. The 96-hour LC_{50} (95 percent) confidence interval (CI) was >1,000 mg/l. Aging the test solutions 7 days did not alter the toxicity to the midge larvae.

Hussain and Oloffs (1980) reported acephate residues in a natural stream following a spray program of acephate to control the western spruce budworm. Aquatic insect larvae showed the highest uptake of acephate (Orthene) and the highest conversion from Orthene to Monitor by N-deacetylation. These levels dropped sharply to near 0 to 8 hours. Orthene was taken up from surrounding water and sediment between 40 and 126 percent. For Monitor, levels found were between 14 and 126 percent of the concentration of Orthene in the larvae. Five hours after the spray, Orthene residues were 190.6 parts-per-billion (ppb) in larvae and Monitor residues were 120.8 ppb, but dropped to nondetectable levels in the next few hours. No toxicity was reported in this study.

The U.S. Environmental Protection Agency (U.S. EPA 1981) research on acephate in the marine environment tested both toxicity and persistence. Static acute toxicity and flow-through acute toxicity tests were conducted for 48 hours to determine EC_{50} and 96-hour LC_{50} values respectively. Maximum Acceptable Toxicant Concentrations (MATC) were determined for the mysid shrimp (Mysidopsis bahia) life cycle and using partial life-cycle tests with grass shrimp (Palaemonetes pugio). The substances tested for acute toxicity were Orthene and Ortho 12420 on algae (96 hours), oyster larvae (48 hours), and copepods and shrimp (96 hours). Acute toxicity levels of acephate were the lowest of the pesticides tested. Acephate was relatively nontoxic, with either high EC_{50} values or no observed effect at concentrations tested. The lowest 96-hour LC_{50} for acephate was 3,800 µg/l. Acute static toxicity for the embryo oyster (Crassostrea virginia) (48 hours) EC_{50} was 150,000 µg/l (8,000 to 300,000 µg/l). The pink shrimp (Penaeus duorarum) (96 hours) LC_{50} was >10,000 µg/l. The acute flow-through toxicity for the mysid shrimp (M. bahia) (96 hours) LC_{50} was 7,300 µg/l (300 to 190,000 µg/l). The acute flow-through (96 hours) LC_{50} for pink shrimp (P. duorarum) was 3,800 µg/l (200 to 7,300 µg/l). The average number of offspring per female mysid exposed to concentrations of 2,000 and 4,200 µg/l was significantly less than that of the controls, 4.9 for exposure to 2,000 µg/l, 3.4 for exposure to 4,200 µg/l and 8.4 for the control (U.S. EPA 1981). The maximum acceptable toxicant concentration

(MATC) that produced no significant effect and the lowest concentration in which significant effects were observed was from 580 to 1,400 μg/l, and the application factor limits were 0.09 to 0.19. Chronic toxicity tests showed little difference between the concentrations that produced chronic effects on mysid shrimp and the 96-hour LC_{50} value for this invertebrate.

The U.S. Environmental Protection Agency also reported results from Rabeni and Stanley (1979, in U.S. EPA 1981) on stream biota effects following acephate spraying in Maine. No mortalities were seen from stream biota, but some perturbations in the stream's ecosystem were noted. Drift of benthos was temporarily increased; invertebrate standing crop remained the same; but acephate had less impact on the ecosystem than did other spruce budworm insecticides.

Schoettger and Mauck (1978) also reported acephate as nontoxic to aquatic invertebrates in static laboratory tests on mature scudes (Gammarus pseudolimnaeus) and stonefly naids (Peteronarcys californica). The acute 96-hour toxicity test results in 18 °C water were LC_{50} = >100 mg/l (95 percent CI). No data were given for stoneflies. These authors concluded that Orthene should not pose a significant toxicity hazard to invertebrates.

2.3 Fish

The Schoettger and Mauck (1978) study above also concluded that acephate (Orthene) was safe for fish. The U.S. Fish and Wildlife toxicity testing (Johnson and Finley 1980) found acephate practically nontoxic to freshwater fish. The U.S. Environmental Protection Agency (1981) testing on marine fish also found acephate to be nontoxic to fish, with very high LC_{50}'s reported. Fish toxicity data are summarized in appendix C.

Schoettger and Mauck (1978) tested brook trout (Salvelinus fontinalis) and Atlantic salmon (Salmo salar) in laboratory cultures to determine LC_{50}'s (95 percent CI). Orthene (94 percent technical) was the least toxic of six pesticides tested; up to 100 mg/l did not kill fish within 96 hours. Orthene LC_{50}'s were all >100 mg/l in temperatures of 12 °C, water hardness values from 40 to 320 mg/l (as $CaCO_3$), and pH values from 6.5 to 9.0 for both 94 percent technical and 75 percent field formulations of acephate.

Johnson and Finley (1980) found acephate to be practically nontoxic to cutthroat and rainbow trout, channel catfish, bluegill, and yellow perch. The most sensitive species to the 94 percent technical acephate was the yellow perch, with an LC_{50} >50 mg/l (slightly toxic). For two trout species (cutthroat and brook) the 96-hour LC_{50} (95 percent CI) was >1,000 mg/l. For the rainbow trout, fathead minnow, channel catfish and bluegill the LC_{50}'s (96 hours) (95 percent CI) were all >1,000 mg/l. The 75 percent soluble powder acephate 96 hour LC_{50} (95 percent CI) was

>100 mg/l for cutthroat and rainbow trout, channel catfish, bluegill, and yellow perch.

In marine fish (U.S. EPA 1981) toxicity results were similar. The acute toxicity (96 hour static culture) for the sheephead minnow (*Cyprinodon variegatus*) LC_{50} was >3,200 mg/l; for the spot (*Leiostomus xantnrus*), >100 mg/l; and for pinfish (*Lagodon rhomboides*), 85 mg/l (7.8 to 33 mg/l). In sheephead minnows, the EC_{50} (96 hours) was set at about 910 mg/l, because one fish died at 1,100 mg/l and some lost equilibrium. The MATC was established as between 0.580 and 1.4 mg/l.

Other investigators also report that acephate is relatively nontoxic to fish (Klaverkamp et al. 1975, Miyaska and Kabayashi 1974, Duangsawaski 1977, Lyons et al. 1976, in U.S. EPA 1981). Duangsawaski (1977, in U.S. EPA 1981) noted inhibition of the ChE activity, decreased heart rate, and increased ventilation rate resulted from acephate applications to waters with rainbow trout, suggesting the heart and respiratory systems as sites for toxicity effects.

Klaverkamp et al. (1975) determined the acute lethality and *in vitro* brain ChE inhibition for acephate. In this study, 110 rainbow trout were distributed in 11 bioassay test vessels. A flow-through system maintained five concentrations of acephate for a 24-hour LC_{50} determination. Acephate concentrations ranged from 3.8 to 1,050 mg/l; pH values were neutral; 15 °C temperatures and dissolved oxygen (DO) levels of 8.7 to 8.8 were maintained. The acute 24-hour LC_{50} for acephate was 895 mg/l and 1,050 mg/l in two tests. Five experiments determined the *in vitro* 50 percent brain ChE inhibition to be at concentrations of 1.4×10^{-1} M. The 24-hour LC_{50} was about 240 times greater than that for fenitrothion. The ChE inhibition was low and did not explain the differences in acute lethality.

Klaverkamp and Hoabden (1980) followed up the above study with an attempt to show how the lethal doses acted in rainbow trout. Concentrations of acephate required to produce 50 percent inhibition of acetylcholinesterase (AChE) was about 125 mmol/l. The lethal doses were apparently not due to brain AChE inhibitory potency.

Watson et al. (1982) believed the effects on rainbow trout could be related to gill and red blood cell disturbance. They tested acephate *in vitro* at concentrations of 100 mg/l to 50,000 mg/l using technical grade acephate solutions. Carbonic anhydrase (CA) activity in blood and gills was significantly ($p < 0.05$) inhibited when acephate concentration was greater than 100 mg/l. Concentrations required to reduce 50 percent CA activity in the gill and red blood cell preparations were 38,000 mg/l and 8,900 mg/l, respectively. The toxic action of acephate may be related to inhibition of CA activity in blood and gills with resultant disturbance of respiratory capacity and salt balance.

In the field, however, acephate had only minor effects on stream fishes and invertebrates (Rabeni and Stanley 1979). Following the spray, fish changed diets to take advantage of the numerous terrestrial species of insects washing into streams, but that effect was temporary. Meanwhile, the benthos of the stream remained unaffected. One species of fish (suckers) was physiologically affected by depressed AchE activity. It was evident in this study that acephate had less impact on aquatic ecosystems than other insecticides used for spruce budworm control.

Geen et al. (1981) and Hussain and Oloffs (1980) reported following an acephate release program, Orthene was not very toxic to fish (LD_{50} of about 2,800 ppm), yet it was moderately persistent in water and may have had effects on fish foods. Ten fingerling rainbow trout and 50 insect larvae were placed in cages in a small stream at several locations downstream of a release site for acephate (Orthene) at a rate of 1,000 ppb. No mortality in either the trout or insect larvae were observed 5 hours later. Orthene residues remained high in water for 5 to 6 hours, then decreased significantly. After 96 hours, the residue in water was near 0. No Monitor was observed in water but was found in fish. Fish converted about 4 to 8 percent of the Orthene to Monitor. Uptake from water to fish tissue was low (1 to 4 percent). However, within 24 hours both Orthene and Monitor were cleared from tissues. Aquatic insects showed the highest Orthene uptake and conversion to Monitor, but these levels dropped to 0 to 8 hours.

Verma et al. (1981) evaluated the toxicity of commercial grade Monitor and commercial and technical grade Orthene to fish (*Mystus vittatus*) for 24, 48, 72, and 96 hours. Both 75.5 and 97 percent acephate were used in this laboratory study in India. Monitor LC_{50} values ranged from 72.8 mg/l for 24 hours to 68.8 mg/l for 96 hours, with a safe concentration of 1.65 mg/l. Commercial Orthene LC_{50} values ranged from 159.4 mg/l for 24 hours to 155.4 for 96 hours, with a safe concentration of 3.783 mg/l. The technical Orthene LC_{50} values ranged from 1,705 mg/l for 24 hours to 1,592 mg/l for 96 hours, with a safe concentration of 38.2 mg/l. Relative potencies of the three pesticides for fish were reported as 1.0 for technical Orthene, 10.24 for commercial Orthene, and 23.14 for commercial Monitor.

Amphibians were surveyed by Buckner and McLeod (1975 in Lambert 1983) as part of a Canadian spruce budworm program. Application rates were 0.5 and 1 lb a.i./acre. It was concluded that amphibians, either in the larval or adult stages, were not affected by the acephate applications. A subsequent study, however, showed that though certain amphibians may not be killed by normal application rates of acephate, bioaccumulations by the amphibians may occur that could threaten other nontarget organisms. Preliminary screening using tadpoles indicated that acephate did not bioaccumulate to levels that threatened mallard ducklings (compared with fenthion and parathion, which bioaccumulated to beyond lethal levels).

Further work by Lyons et al. (1976, in Lambert 1983) indicated that tadpoles do accumulate acephate in their body tissues, but only to maximum concentrations approximately equal to acephate in the surrounding water. They estimated that, at maximum body concentrations anticipated from normal application rates, an adult mallard would have to consume 4.7 x 10^6 tadpoles to accumulate lethal levels of acephate. This does not rule out possible sublethal effects such as changes in ChE activity, but it does indicate a wide margin of safety to certain tadpole predators (Lambert 1983).

2.4 Birds

Acephate is moderately toxic to birds but often results in sublethal reductions in brain ChE activity temporarily following a spray. Birds are more sensitive to acephate by oral or inhalation doses than are mammals.

Berteau and Chiles (1978) compared the toxicity of acephate to rats, mice, and quail and found that quail are more susceptible than rodents to acephate when it is administered as an aerosol. Respirable aerosols of aqueous formulations of acephate (2.2 mg/l) were provided for a 5-hour exposure. Three of the 6 quail died after 100 minute exposures to the aerosol. Extent of plasma ChE depression was compatible with mortality incidence. Recuperation from the acephate dose was slow (no time given).

Zinkl et al. (1980, 1981, 1984) reported the results of acephate on wild bird populations, with particular interest in the sublethal measurements of ChE inhibition in the brain. Fleming and Bradbury (1981) reported the inhibition of ChE activity is the primary mode of action for organophosphorus pesticides.

Zinkl et al. (1980) reported inhibition of brain ChE activity in birds and squirrels exposed to acephate applied aerially for control of western spruce budworm. Acephate was applied at a rate equivalent to 0.5 lb/acre (0.57 kg/ha), and individuals of various species of birds and squirrels were collected from the field up to 26 days after spray application. Among 11 bird species sampled, 11 of 22 individuals had depressed ChE on the day of spray; 14 of 26 showed depression at 1 day; 13 of 14 at 3 days; 18 of 27 at 6 days; 3 of 19 at 25 to 26 days; maximum depression of 57 percent in chipping sparrow was reported on day 6. Authors postulated that since birds with continuous exposure will die at 50 percent ChE inhibition, this exposure may be life threatening. However, at application rates of 0.57 kg/ha, no mortality of birds was observed, nor were there signs of oroganophosphate insecticide poisoning in the field. Also, no residues were found in the birds with ChE depression.

Zinkl et al. (1981) followed up on a study of acephate toxicity to dark-eyed juncos (Junco hyemalis). This study indicated that the amount of acephate needed to produce the ChE depression was about one-fifth of the LD_{50} value; however, in a few birds the ChE activity may be depressed to near lethal levels. When acephate (Orthene 75 SP) was applied at a rate of 1.13 kg/ha (1 lb/acre), passerine birds exhibited depressed brain ChE levels up to 33 days following the spray. Zinkl et al. (1981) suspected that either acephate or its metabolite methamidophos caused the ChE depression, which can be lethal to some birds. Juncos in a laboratory were given a single oral does of acephate and methamidophos at doses of 37.5, 75, 106, 150, 212, and 300 mg/kg to determine the LD_{50} and brain residue concentrations, to see if these correlated with ChE depression. The acute oral LD_{50} for acephate was 106 mg/kg (moderately toxic) and for methamidophos 8 mg/kg (very highly toxic). Birds were also fed acephate for 5 days at levels ranging from 625 to 5,000 mg/kg. The LD_{50} was 1,495 mg/kg (probit level 1,500 ppm), which is considered to be slightly toxic. Brain ChE activity in birds that died after acephate doses was depressed 80 percent below that of the controls. Birds that died from methamidophos had brain ChE depression of 60 percent. The depression of ChE to 50 percent or more can be lethal to birds. For the 5-day feeding, the ChE levels of birds that died early in the study were less depressed (51.5 percent) than those that died at a later date (69.6 percent). Survivors returned to normal within 3 days. These authors suggested that a compound other than acephate or methamidophos may inhibit the brain ChE. The magnitude of ChE depression in birds in the field could not be explained by the doses they would receive in normal spray programs. ChE activity is a better indicator of the effects than residue analysis following a spray.

Although wild birds may suffer sublethal effects from acephate, it is unlikely that rates applied at 0.85 kg/ha or less will kill many birds (Zinkl et al. 1984). This rate was used for suppression of pandora moths (Coloradia pandora), and brain ChE depression in six species of wild birds was measured following the spray: grey-headed juncos (Junco caniceps), western bluebirds (Sialia mexicana), Grace's warblers (Dendroica graciae), yellow-rumped warblers (D. coronata), pygmy nuthatches (Sitta pygmaea), and American robins (Turdus migratorius). Acephate and its metabolite methamidophos residues in brain and liver were highest on the day of the spray and decreased to undetectable concentrations by 14 days. Brain ChE was not inhibited on day 1 but was significantly inhibited ($p = 0.05$) on day 2 after the spray. There was no correlation between brain ChE activity and residues found in the brain or liver. Booth et al. (1977, in Zinkl et al. 1984) concluded that a bird would have to consume several times its body weight of contaminated food for either acephate or metamidophos to depress brain ChE activity to a level found in birds from forest application of acephate. Zinkl et al. (1984) concluded that after application of 0.57 to 2.26 kg/ha acephate (0.5 to 2.0 lb/ac) it would be difficult for a bird to ingest or absorb sufficient acephate to produce 20 to 50 percent brain ChE inhibition. Birds would be expected to recover from acephate doses. In vivo recovery of brain ChE activity occurred within 8 days from dosing with an LD_{50} level (Fleming and Bradbury 1981). Another unknown compound may be formed from acephate. It is unlikely that large numbers of birds would be killed by acephate spray programs.

Orthene was used experimentally in a series of tests against spruce budworms at two sites in eastern Canada (Buckner et al. 1975, in Lambert 1983). Application rates were 0.5 and 1.0 lb a.i./acre. Actual application rates, measured at ground level, ranged from 0.09 to 6.5 lb a.i./acre. Adverse effects were monitored through changes in songbird populations and by noting the presence of obviously ill or dead birds. Daily surveys conducted 5 days before spraying through 6 days after spray found that the songbird populations monitored were not adversely affected by the acephate applications.

Julin et al. (1977, in Lambert 1983) reported on the effects of a controlled application of 0.5 lb a.i./acre of acephate on songbirds inhabiting a 60,000-acre spruce-fir-hardwood forest block in Maine. Birds of interest were evening grosbeaks, redstarts, magnolia warblers, and Canada warblers. Observations and samples of the songbirds were taken through 35 days after spraying. A search of the spray block in conjunction with sampling produced no obviously affected songbirds or other vertebrates. By the final sampling, before spraying brain ChE activity levels were approached in all species. There was, however, significant depression of brain ChE activity was noted in evening grosbeaks (maximum 18.1 percent at 1 day after spraying) and magnolia warblers (maximum 22.8 percent at 10 days after spraying). There was also observed a high degree of variation in brain ChE both within and between species. This may result from differences in behavior.

Acephate was used in a comparative study to assess effects of application rates of 1 and 2 lb a.i./acre on nontarget nesting bird populations in the forests of northeastern Oregon. Inhibition of ChE activity was used to measure organophosphate intoxication. Richmond et al. (1979, in Lambert 1983) reported that acephate caused extensive depression of brain ChE activity with decreases of 3 to 50 percent recorded for 11 of 12 species collected after spraying. On the plot treated with 1 lb a.i./acre, 45 of 65 birds collected 1 to 33 days following spraying had decreased ChE activity. The highest frequency of depression was noted on the second day after spraying.

Bart (1979, in Lambert 1983) reported a comparative study between acephate and other insecticides on two forested plots in northeastern New York. Acephate was applied at rates of 0.5 lb. a.i./acre. Songbird surveys were made before and after spraying using a signing-male mapping technique. Following the acephate application, a significant decline occurred in the number of red-eyed vireos on the treated plot. It was not determined whether the decline was due to direct effects or to indirect effects such as depletion of their food supply.

A single dose of acephate (98 percent pure) was given orally to mallard ducklings, at a rate of 1,350 mg/kg, an amount determined to reduce ChE by 40 to 50 percent. Acephate caused death of 11 of 36 ducklings. Decreased ChE activity at death was significantly different among organophosphorus pesticides: fensulfothion > parathion > acephate > malathion. After dosing within 24 hours, plasma ChE activity was depressed 90 percent and brain ChE activity was depressed 48 percent for acephate. There was a wide range of sensitivity to pesticides tested,and these authors concluded it is not likely that uniform ChE depression will be found in birds from recent spray areas.

Hoffman and Albers (1984) dipped mallard eggs in acephate (Orthene) to measure LD_{50}'s of insecticides in aqueous emulsion by egg immersion to compare toxicities and teratogenic effects and impaired embryonic growth. The LC_{50} for acephate was 54 (41 to 71) lb/acre at 100 gal/acre (65.9 kg/ha) (95 percent CI). Significant differences ($p < 0.05$) were noted for reduced growth, both less than and more than the LD_{50}, and for abnormal survivors, both less than and more than the LD_{50} dose. At day, 18 survivors exhibited the following malformations: bill, edema, eye, neck, stunted (sic). Among the 14 insecticides tested, acephate was less toxic than endrin, sulprofos, and parathion but more toxic than 10 others. However, when the authors compared the highest permissible field levels for each, acephate was among the four with smallest potential hazard.

Wildlife International Ltd. (1985) reported that chickens exposed to acephate technical aqueous solutions of 785 mg/kg (equivalent to formulation concentrations of 196 mg/g) exhibited no signs of delayed neurotoxicity and had no lesions.

Summaries of bird toxicity data can be found in appendix D.

2.5 Mammals

Toxicity studies of the effects of acephate in humans and in laboratory mammals including rats, mice, guinea pigs, rabbits, and dogs are summarized in table 2-1 and in appendix E. Results of these studies are discussed below.

Acephate is moderately to slightly toxic to mammals by the oral route (LD_{50}'s range from 233 to 1,800 mg/kg) and slightly toxic to mammals exposed by the dermal route (LD_{50}'s range from 2,000 to >10,250 mg/kg). Technical grade acephate is somewhat less toxic to mice than purified acephate, and storage of technical acephate (at 20 or 40 °C) decreases its mammalian acute toxicity but does not alter its toxicity to houseflies.

Subchronic oral doses of acephate to rats results in inhibition of ChE activity, an increase in total liver lipid, and changes in the activity of some hepatic enzymes (i.e., succinic dehydrogenase and lactic dehydrogenase). No other systemic effects have been reported, and no subchronic toxicity studies on acephate administered by other routes have been identified.

Chronic oral exposure to acephate results in a decrease in ChE activity as indicated by studies in rats and dogs. The NOEL for this effect was 0.25 mg/kg for rats and 0.75 mg/kg for dogs (U.S. EPA 1983b). However, no adverse systemic toxicity effects and no blood ChE inhibition were detected in workers chemically exposed to acephate even though acephate was detected in their urine at levels up to 5 ppm.

No teratogenic effects have been detected in rats and rabbits after administration of acephate (route unspecified).

Based on the toxic effects observed following acute, subchronic, and chronic exposure to acephate, this insecticide is an inhibitor of ChE activity and would be expected to result in neurotoxic effects in mammals.

No bioassays to determine the carcinogenic potential of acephate were identified, and it is assumed that there have been bioassays performed on acephate. The mutagenic potential of acephate has been studied in bacterial assays, yeast cell assays, mammalian cell _in vitro_ assays, and _in vivo_ assays using mice and fruit flies. Acephate has exhibited mutagenic activity in bacterial reverse-point mutation assays (specifically, those that detect base-pair substitution events), yeast cell assays, and mammalian cell _in vitro_ assays. Therefore, since acephate has mutagenic activity, there is limited support for concern that this insecticide may have carcinogenic potential.

2.5.1 Acute Toxicity

The toxic effects associated with acute exposure to acephate by the oral route have been studied in the rat, mouse, and dog (Registry of Toxic Effects of Chemical Substances 1981-1982, Meister 1984, U.S. EPA 1983b, Fukuto 1983). The toxic effects of acute exposure to acephate by the dermal route has been studied in rabbits (Meister 1984). There were no acute toxicity studies of acephate administered by ocular or inhalational routes identified. The oral LD_{50}'s for acephate ranged from 233 mg/kg in mice up to 1,800 mg/kg in rats. The dermal LD_{50}'s ranged from 2,000 to

>10,250 mg/kg in rabbits. Using the U.S. Environmental Protection Agency toxicology guidelines, acephate is in toxicity categories I to II (moderately to slightly toxic) for oral toxicity and category III (slightly toxic) for dermal irritation.

Fukuto (1983) reported the most complete study of the acute oral toxicity of acephate. An important finding of this study was that some of the impurities frequently present in this insecticide have an antagonizing effect on its mammalian toxicity. In a previous article, Kao and Fukuto (1977) postulated that the low mammalian toxicity of acephate may be due to this insecticide's ability to avoid hydrolytic activation to the more-effective anticholinesterase, methamidophos, also a more toxic insecticide. It was further postulated that impurities in technical grade acephate interfere with the esterase cleavage of the N-acyl linkage of acephate to yield methamidophos. Fukuto (1983) found the rat oral LD_{50} of technical grade acephate was 1,800 mg/kg, while the oral LD_{50} of purified acephate was 1,480 mg/kg. In mice, the oral LD_{50} of technical grade acephate was 1,800 mg/kg, while the oral LD_{50} of purified acephate was 425 mg/kg. The oral LD_{50} in mice of purified acephate plus 5 percent impurities (unspecified) was 520 mg/kg. The specific impurities found by Fukuto (1983) in technical grade acephate were O,O-dimethyl-N-acetyl-phosphoramidothioate, O,O,S-trimethyl phosphorodithioate (TMP), and methamidophos. In one experiment with mice that was reported by Fukuto (1983), the oral LD_{50} of purified acephate was 570 mg/kg while (1) the LD_{50} of acephate plus 1 percent O,O-dimethyl-N-acetylphosporamidothioate was 720 mg/kg, (2) the LD_{50}'s of acephate plus 0.5 and 1 percent TMP were 540 and 460 mg/kg, respectively, and (3) the LD_{50} of acephate plus 5 percent methamidophos was 570 mg/kg. Therefore, O,O-dimethyl-N-acetyl-phosphoramidothioate caused a significant decrease in mouse toxicity while TMP caused a slight potentiation of the toxicity of acephate. The former impurity was considered by the author to be the major reason for the lower acute oral toxicity of technical grade acephate compared to the purified material. However, when technical grade acephate was stored at 40 °C for 6 months, its mouse oral LD_{50} decreased from 500 mg/kg (before storage) to 820 mg/kg after 6 months and the toxicity of this acephate preparation to houseflies was unaltered. Since the concentration of O,O-dimethyl-N-acetylphosporamidothioate decreased from 0.44 to 0.27 percent, this impurity of technical grade acephate is not the complete explanation of the lower mammalian toxicity of technical grade acephate.

2.5.2 Subchronic Toxicity

Subchronic oral toxicity of acephate has been studied in rats. The toxic effects observed following oral administration of acephate to rats are inhibition of ChE activity and changes in the activity of some hepatic enzymes. No other systemic toxicity effects have been reported. Although studies of the subchronic oral toxicity of acephate have been reported, studies of the subchronic toxicity of acephate administered to mammals by other routes have not been identified.

According to the U.S. Environmental Protection Agency (1983b), the oral no observable effect level (NOEL) of acephate in rats is 0.25 mg/kg based on the inhibition of ChE activity in plasma, red blood cells, and brain tissues of rats fed acephate in food for 90 days. No details of this study were reported.

Brorby (1987) reported a study in which groups of 30 adult Sprague-Dawley rats of each sex were fed diets containing 0, 2, 5, 10, and 150 ppm acephate technical grade. Subgroups of ten animals/sex/group were killed and examined after 4, 9, and 13 weeks. No mortality or signs of toxicity related to treatment were observed throughout the study. AChE activity was significantly lower only in males fed the 150-ppm diet 4 and 9 weeks and in females at 9 and 13 weeks. The only statistically significant decrease in BChE activity was in females fed the 150-ppm diet at 13 weeks. It was also reported that rat brain AChE has been shown to be unusually sensitive to acephate as compared to monkey and human brain.

Choudhari and Chakrabarti (1984) reported a study in which albino rats were given oral doses of 600 mg/kg/day of acephate (75 SP). The acephate, (oral LD_{50} in rats of 1,494 mg/kg) was dissolved in distilled water. Animals from an untreated control group and the acephate-treated group were deprived of food for 18 hours before they were killed at the end of 2, 4, 6, and 8 weeks. After 4, 6, and 8 weeks of administration of acephate, there was a slight increase in total liver lipid or triglycerides. Preceeding and accompanying the increase in lipid triglycerides, there was a decrease of plasma triglycerides. Because triglycerides are secreted from the liver primarily in the form of lipoprotein, an acephate-induced impairment in the synthesis and/or release of lipoprotein was postulated by the authors. No other systemic toxicity effects of oral administration of acephate to rats were reported.

In a study by Patel and Chakrabarti (1982), albino rats were given oral doses of acephate at 60 mg/rat/day for 30, 45, or 60 days. Since rats weighed 100 to 150 g each, the daily dose ranged from 400 to 600 mg/kg/day. After administration of acephate for 30, 45, or 60 days, the activity of liver succinic dehydrogenase (SDH), lactic dehydrogenase (LDH), and the levels of liver and blood glutathione (GSH) were measured. The activity of SDH in liver and the level of GSH in liver and blood were decreased following acephate treatment. The decline in the activity of SDH was dose elated and most pronounced in animals treated for 60 days (SDH activity 53 percent of control level). The activity of LDH in the liver of treated rats was significantly elevated ($p > 0.05$) in animals treated with acephate; up to an 8 percent increase in activity was observed in rats treated for

60 days. The liver and blood GSH levels were found to be significantly reduced ($p < 0.02$; liver GSH was 33 percent of control level) after 60 days of treatment with acephate.

2.5.3 Chronic Toxicity

The effect on ChE activity of chronic exposure to acephate has been studied in rats, dogs, and humans. While the details of some of these studies are not available, ingestion of acephate resulted in inhibition of ChE activity in rats and dogs. Dogs apparently exhibited systemic toxicity effects after chronic exposure to acephate, but details were not available. However, no inhibition of ChE activity was detected in acephate-exposed workers.

The U.S. Environmental Protection Agency (1983b) reported that rats fed acephate (dose unspecified) for 28 months, exhibited inhibition of ChE activity in plasma, red blood cells, and brain tissue. The NOEL for this effect in rats was 0.25 mg/kg (5 ppm in food). No additional details of the study were reported.

According to the U.S. Environmental Protection Agency (1983b), dogs given acephate (dose unspecified) in their food for 2 years exhibited inhibition of ChE activity in plasma, red blood cells, and brain tissue. The NOEL for the inhibition of ChE activity in dogs was reported to be 0.75 mg/kg (30 ppm in food). It was also reported that the NOEL for systemic toxicity of acephate in dogs is 2.5 mg/kg (100 ppm in food). Although there are no additional details of this study available, chronic exposure to acephate apparently results in unspecified systemic toxicity effects in dogs.

Occupational exposure to acephate at both a manufacturing facility and in a laboratory that formulated large batches of acephate resulted in workers having urine levels of acephate up to 5 ppm (Willcox and Coffey 1977, in USDA Forest Service 1984). However, no adverse lethal effects were observed, and no inhibition in blood ChE activity was detected in these workers. It should be noted that blood ChE activity levels are considered a sensitive indication of organophosphate exposure.

2.5.4 Reproductive and Teratogenic Toxicity

Hoberman (1987) reported on the results of a reproduction study in rats using acephate, technical grade. The NOEL for both the adult and litter (developmental) stages was 50 ppm. The study used dietary levels of 0, 25, 50, and 500 ppm acephate. Even at the highest level, there were no effects on mating or pregnancy incidences nor did histopathological examination reveal any lesions due to acephate administration. At the

500 ppm level, however, certain effects were exhibited: low body weight despite increased feed consumption; significant decrease in implantation for the third generation dams; and significant decreases to live litter size for the second litter of the first generation, both litters of the second generation, and the single litter of the third generation.

There is no data to indicate that acephate induces teratogenic toxicity effects. According to the U.S. Environmental Protection Agency (1983b), the NOEL for teratogenic effects is ≥ 200 mg/kg (highest dose tested in rats and ≥ 10 mg/kg (highest dose tested) in dogs. No additional information on either of these studies is available.

2.5.5 Neurotoxicity

There were no neurotoxicity studies of acephate in mammals identified, and it is assumed that none are available. Therefore, no neurotoxicity data are presented in appendix F. Based on the toxic effects observed following acute, subchronic, and chronic exposure to acephate, this insecticide is an inhibitor of ChE activity and would be expected to exhibit neurotoxic effects in mammals.

2.5.6 Carcinogenicity

There were no carcinogenicity bioassays of acephate in mammals identified, and it is assumed that none are available. The U.S. Environmental Protection Agency (1983b) reports acephate is not carcinogenic. Therefore, no carcinogenicity data are presented in appendix F.

2.6 Mutagenicity

The mutagenic effects of acephate have been reviewed by Waters et al. (1980). Summaries of studies on the mutagenic activity of acephate are presented in table 2-1 and appendix G. The mutagenic potential of acephate has been tested in bacterial assays, yeast assays, *in vitro* mammalian cell assays, and *in vivo* in mice and in *Drosophila melanogaster*.

Acephate has exhibited mutagenic activity in bacterial cell assays, yeast assays, and *in vitro* mammalian cell assays.

Bacterial (prokaryotic cell) mutagenicity assays are designed to detect point (i.e., singe gene) mutational events. The Ames test developed in 1975 used selected strains of *Salmonella typhimurium* to detect mutagenic activity of chemicals in the presence and absence of metabolic activation by a preparation of mammalian (usually rat) liver enzymes referred to as S9. Use of different strains of *S. typhimurium* in the Ames assay allows determination of whether reversion of an existing point mutation resulted from a frameshift or a base-pair substitution event. Acephate has been demonstrated to be mutagenically active in strain TA100 of *S. typhimurium* to detect mutagenic activity of chemicals in the presence and absence of metabolic activation by a preparation of mammalian (usually rat) liver enzymes referred to as S9. Use of different strains of *S. typhimurium* in the Ames assay allow determination of whether reversion of an existing point mutation resulted from a frameshift or a base-pair substitution event. Acephate has been demonstrated to be mutagenically active in strain TA100 of *S. typhimurium*, which detects base-pair substitution events (Shirasu et al. 1981, Waters et al. 1980, Moriya et al. 1983). Although not reported by Shirasu et al. (1981) or Waters et al. (1980), the report by Moriya et al. (1983) indicated that acephate was mutagenically active in strain TA100 both with and without S9 metabolic activation, which indicates that acephate is a direct-acting mutagen (i.e., does not require biotransformation to an active form) in this system. *S. typhimurium*/Ames tester strains less sensitive to mutational base-pair substitution events have not detected mutagenic activity in the acephate preparations tested (Waters et al. 1980, Moriya et al. 1983, and Hanna and Dyer 1975). In addition, Seiler (1977) reported that acephate, even after being nitrosated *in vitro* with sodium nitrate, did not induce a significant increase in revertants (reverse-point mutational events) *S. typhimurium* strain hisG46>. Nitrosated acephate was tested without S9 metabolic activation at 1, 10, and 100 µl/plate using the spot test method.

S. typhimurium has also been used to detect mutagenic activity in a differential toxicity assay. This assay is based on increased susceptibility of DNA-repair-deficient strains of bacteria to chemical toxicity over that of DNA-repair-proficient strains. Waters et al. (1982) reported that acephate was not mutagenic in a differential toxicity test using *S. typhimurium* tester strains S14525, which is DNA-repair-proficient (rec^+), and SL4700, which is DNA repair-deficient (rec^-). Details of this study were not reported.

Assays using selected strains of *Escherichia coli* have also been used to test acephate for mutagenic activity. Hanna and Dyer (1975) reported that acephate (Orthene 75 SP) tested in the presence and absence of S9 metabolic activation exhibited mutagenic activity in an *E. coli* reverse-point mutation spot test. Although all of the *E. coli* strains used by Hanna and Dyer (1975) are used to detect mutational base-pair substitution events, strains WP2, WP2uvrA, WP67, and WP12 of *E. coli*, but not strains CM561, CM571, and CM611, were sensitive to (responded to) the mutagenic activity of acephate. Moriya et al. (1983) also reported that acephate is mutagenically active in *E. coli* strain WP2 her, which also detects base-pair substitution events. No details of the study by Moriya et al. (1983) were available. The only *E. coli* reverse point mutation assay that did not detect acephate mutagenic activity was reported by Waters et al. (1980). While *E. coli* strain WP2 has been reported to be responsive to acephate mutagenic activity (above), it is possible that the unspecified dose levels tested by Waters et al. (1980) may have been inadequate to induce a detectable response.

Waters et al. (1980) reported that acephate had been tested in two differential toxicity assays; one using *E. coli* POL A strains W3110 (rec$^+$) and P3478 (rec$^-$) and one using *Bacillus subtillus* strains H17 (rec$^+$) and M45 (rec$^-$). In the 1980 report of these studies, Waters et al. (1980) concluded that acephate was not mutagenically active in this assay. Subsequent to that initial report, Waters et al. (1982) concluded that the results of both assays were inconclusive because acephate did not inhibit the growth of ether strain species of bacteria. The authors speculated that acephate may lack the ability to diffuse into agar and, therefore, may not have had access to the bacteria.

Yeast assays, which can be used to detect mitotic recombination, crossing over, mitotic gene conversion, and reverse mutation, have also been used to evaluate the mutagenic potential of acephate. *Saccharomyces cerevisiae* strain D3 is used to detect mitotic recombination *S. cerevisiae* strain D7 is used to detect induction of mitotic crossing over, mitotic gene conversion, and reverse-point mutation. Riccio et al. (1981), who did not report any details of the study (e.g., dose levels, resultant data, etc.), stated that acephate induced mitotic recombination of *S. cerevisiae* strain D3 and mitotic crossing over, mitotic gene conversion, and reverse-point mutation in the strain D7 assay. Waters et al. (1980) also reported that acephate induced mitotic recombination in *S. cerevisiae* strain D3. In addition, Waters et al. (1982) confirmed that acephate was mutagenically active in *S. cerevisiae* strain D7. Although study details were not reported by Waters et al. (1982), acephate with, but not without, S9 metabolic activation induced mitotic gene conversion, crossing over, and reverse-point mutation in *S. cerevisiae* strain D7.

Assay using mammalian cells *in vitro* have also been used to evaluate the mutagenic potential of acephate. Waters et al. (1980) reported that unspecified levels of acephate in the absence of S9 metabolic activation increased unscheduled DNA synthesis (UDS) in human fetal lung fibroblasts (WI-38) over untreated control values. In the presence of S9 metabolic activation, acephate increased unscheduled DNA synthesis (UDS) in human fetal lung fibroblasts (WI-38) over that in untreated controls. In the presence of S9 metabolic activation, acephate did not induce UDS in WI-38 cells. Acephate has also exhibited mutagenic activity in mouse lymphoma L5178Y cells and induced sister chromatid exchange levels significantly above spontaneous control levels in Chinese hamster ovary (CHO) cells (Waters at al. 1982). Details of these studies are not available.

In vivo tests of the mutagenic potential of acephate have been conducted in mice (Waters et al. 1982) and Drosophila melanogaster (Valencia 1977, Waters et al. 1980). Waters et al. (1982) reported that unspecified dose levels of acephate did not induce a significant increase in micronuclei in mouse red blood cells. Details of this study were not reported. Valencia (1977) reported on the results of a sex-linked recessive lethal assay in which 10 ppm acephate was fed to male D. melanogaster for 48 hours before mating them at successive intervals of 4, 3, 3, and 4 days to produce four broods of progeny. The response in the acephate-treated group of fruit flies was not significantly different from control values and acephate was concluded to be negative for mutagenic activity in this assay. This result was confirmed by Waters et al. (1980), who also reported that acephate produced negative results in the sex-linked recessive lethal assay. While details of the D. melanogaster sex-linked recessive assay test conducted by Waters et al. (1980) were not available, it is apparent that acephate was fed to males at a dose of 10 ppm for at least 24 hours before mating.

3.0 Environmental Fate

Lambert (1983) reports acephate is classified as a relatively nonpersistent organophosphorus insecticide. Compared with most other insecticides, it has the following characteristics:

- Short half-life in soil, probably due to relatively high biodegradability.
- High soil mobility with little retention in soils.
- Moderate persistence in natural waters.
- Moderate persistence in leaves.
- No bioaccumulation in food-chain organisms, but some uptake by aquatic organisms during direct exposure.

Literature is incomplete on the fate of acephate in the biotic and abiotic environment. Acephate is stable with residual systemic activity of approximately 10 to 15 days in plants. A small percentage of acephate in the biota is converted by N-deacetylation to methamidophos, which is more toxic in the environment. In soils, acephate is biodegradable and not harmful to microorganisms, but it is persistent when applied as a soil drench. Since it is water soluble, acephate has potential to leach into water. Acephate is reported to degrade in a few days on fruit, but to last 2 to 4 weeks on foliage. The manufacturer recommends a 21-day safety factor prior to harvest of crops. Residues may persist up to 7 days in birds, 96 hours in water, and 24 hours in benthos, insects, and sediments, and 3 weeks in soil.

Although acephate has low toxicity to fish, it may alter feeding habits temporarily. Acephate is not accumulated significantly in fish or frogs but is taken up and converted to the more toxic methamidophos by both fish and invertebrates. There is some indication of potential effects on the food chain in soil microorganisms and benthos, but specific studies to measure bioconcentration factors are lacking.

3.1 Bioaccumulation and Metabolism

3.1.1 Plants

Lambert (1983) reports acephate as having a relatively low rainfastness. If applied to wet trees it has a significantly lower effectiveness compared to application to dry trees. It is degraded in performance by the presence of water before or after application. A decay half-life was reported as 3 to 6 days with no residue of acephate or methamidophos after 60 days (Szeto et al. 1978). Plants readily absorb acephate from leaves and from the soil, transport it via the xylem and accumulate it in leaves. The degradation of acephate on plants is thought to follow a common pattern. That fraction of insecticide that is not absorbed by plant tissue immediately upon application is subject to washoff and other mechanisms for degradation, transport from the plant, and chemical/ microbiological breakdown. That fraction of the insecticide that is absorbed by the plant has 5 to 10 percent of its concentration metabolically transformed to methamidophos. Both the remaining acephate and methamidophos are metabolically degraded over time to innocuous salts (Lambert 1983).

Table 3-1 presents a summary of the literature on residues and residence time for acephate on plants. After 24 hours, 40 percent of the acephate remained in leaves (Bouchard and Lavy 1982). By 48 hours, greater than 50 percent was absorbed by leaves and about 9 percent was metabolized to methamidophos (Bull 1979). Concentration peaked between 3 and 21 hours following an acephate spray, declined by 50 percent in 3 days, and to less than 1 percent in 45 days; none was detectable in Douglas-fir needles after 60 days (Szeto et al. 1978). Most authors report that the residence time for acephate in plants is 22 days or less (Bull 1979, Bouchard and Lavy 1982, Oetting et al. 1984, Linquist and Krueger 1975, Leidy et al. 1978, Richmond et al. 1978, Mowlam et al. 1977, Nigg et al. 1979 and 1981, Leidy and Sheets 1978). Fitzpatrick and Bogan (1980) estimated the foliar half-life of 8.4 to 8.9 days, with a residue of greater than 1 ppm lasting 1 month on citrus leaves. For tobacco, the curing process caused loss of 97 percent of the acephate residue (Leidy and Sheets 1978). Werner (1974) treated southern pine seeds with acephate (92 percent a.i.) for 24 to 48 hours (1 to 5 percent solution in water). Four-week-old seedlings retained sufficient quantities (7.5 percent acephate) to kill more than 60 percent of the pales weevil (Hylobius pales). The acephate-treated group of seedlings indicated no adverse effects on growth 6 months after treatment. Monitor, on the other hand, was phytotoxic in seeds, with only about 1/3 germination reported.

Seeds of white spruce and yellow birch were treated with acephate by Pomber and Weinberger (1976, in Lambert 1983). They concluded that acephate had a slight inhibitory effect on the germination speeds of white spruce seeds (10 ppm acephate treatment concentration). In contrast, acephate appears to significantly increase germination speed and final germination percentages of yellow birch seeds. Seedlings of neither species had adverse effects on growth.

Acephate had a more severe effect on the seed germination rates of Pinus halepensis (Olofinboba and Kozlowski 1982, in Lambert 1983). Only half as many seeds germinated when exposed to 5 and 45 g/l treatments of acephate as compared to experimental controls. Seedlings subjected to root

Table 3.1

Summary of Residues of Acephate in Plants

Formulation	Application/Exposure	Plants (Parts)	Residue Levels	Residence Time	Reference
^{14}C-labeled acephate and Monitor	Singular foliar application 10,000 cpm/g	Cotton (leaves)	>50% absorbed by leaves about 9% metabolized to methamidophose metabolites translocated throughout plant	48 hrs	Bull 1979
^{14}C-labeled acephate	Successive foliar application 6 day intervals (3 X)	Cotton (leaves)	40% present in leaves	24 hrs (half-life 8 days)	Bouchard and Levy 1982
Slow-release tablets acephate 2.5%; 75% SP; and 10% granule	Two min. of daily soaking tablets (0.025 to 0.075 g a.i./pot) applied to soils in pots for 7 weeks	Ornamentals in containers, azalea (roots) chrysanthemum	90% recoverable acetate reduced from tablets to soils	15 days	Oetting et al. 1984
Acephate 75 SP	Greenhouse tomatoes 0.08 to 0.12% a.i./plant spray application	Tomato (leaves)	0.08% rate reduced to 18% by 21 days; 0.12% rate reduced to 22% by 21 days. Recoveries for acephate 85 to 93%	21 days	Lindquist and Krueger 1975
Acephate (75% SP)	Greenhouse application 0.56, 1.12, 2.24 kg a.i./930 l/ha; 4 successive weekly applications	Tomato (leaves)	Recovery for acephate 90%; by day 7 residue was 5.4% of day 0 rate	7 days	Leidy et al. 1978
Acephate (Orthene)	Field application	Tomato (fruit)	1 ppm	Not given	U.S. Food and Drug Administration 1980
Acephate (Orthene)	Spray 4.0 g a.i./l at weekly intervals	Bean (fruit) Cabbage (leaves) Squash (fruit)	88 to 89% soil. 93.6% cabbage recovered	1 hr	Narain et al. 1980
Acephate	Application rate 500 ppm	Peach (shoots)	Residue lasted	22 days	Nowlan et al. 1977
Acephate and Methamidophos	Spray backpack 0.6 g a.i./l (about 38 l/tree)	Citrus trees: Temple and Valencia orange, grapefruit, lemon, tangerine (rinds)	Half-lifes: rind 10.3 to 10.5 days; pulp 6.1 to 15 days at 7 days; maximum levels	21 days	Nigg et al. 1979

Table 3-1 (Concluded)

Formulation	Application/Exposure	Plants (Parts)	Residue Levels	Residence Time	Reference
Acephate and Methamidophos (Monitor)	Spray 21-day intervals 0.6 g/l; third application 1.6 g/l by mistblower	Citrus trees (leaves)	Foliar half-life: 8.4 to 8.9 days	>1 ppm, 1 mo	Fitzpatrick and Bogan 1980
Acephate and Methamidophos	Three-week intervals 0.6 g a.i./l (0.5 lb/gal) (10 gal/ tree) by mistblower or handgun	Oranges, lime, grapefruit, lemon, and tangels (leaves)	Foliar half-life: 6.8 days mistblower and 12.6 days by handgun	14 days	Nigg et al. 1981
Acephate and Methamidophos	Emulsified concentrate 0.56 to 1.12 kg/ha flue curing of leaves	Tobacco (leaves) fresh and cured	Total residue on cured leaves 0.20 ppm; curing caused loss of 97% of residue	5 days	Leidy and Sheets 1978
^{14}C-labeled Orthene and Monitor	1 ml mixture Orthene (7.0 mg) and analytical Orthene (11.6 mg) up to 120 hrs	Loblolly pine (seedlings) (roots)	Rapid adsorption and translocation	1 hr	Werner 1974
Acephate (Orthene)	10 to 100 ppm exposure to seeds	White spruce Yellow birch	Twenty-one-day germination study, inhibited spruce, enhanced birch	NA	Weinberger et al. 1978
Acephate (Orthene) (Monitor)	1.12 kg/ha aerial spray	Douglas-fir (needles)	Peak concentration Declined 50% <1% None detected	3 to 21 hrs 3 days 45 days 60 days	Szeto et al. 1978
Orthene 75S	Field application 1 lb a.i./acre	Peanut soil dust	Maximum levels of 0.0257 µg/sq cm	Half-life 8 days	Lai 1987f
Orthene 75S	Field application 1 lb a.i./acre	Cauliflower leaves	Levels ranged from 0 to 0.73 µg/sq cm	Half-life 7.5 days	Lai 1987b

NA = data not available.

dip treatments showed significant losses in stem and needle weights compared to controls 45 days after treatment.

Adult ornamental plants were tested for phytotoxic effects by Neel et. al (1977, in Lambert 1983). Maidenhair ferns, painted fingernail bromeliads, white cattelya orchids, Australian tree ferns, birdsnest ferns, Boston ferns, and staghorn ferns were dosed to runoff with 0.5 and 1.0 lb of acephate 75 WP/100 gal of water at 1-week intervals for 3 weeks. No apparent phytotoxicity was observed, but there was reason to believe that phytotoxicity risk may have been related to the high temperatures.

The systemic transport of acephate was shown by Werner (1974, in Lambert 1983). When 130-day-old loblolly pine seedling roots were emersed in nutrient solutions containing radiolabelled acephate, roots rapidly absorbed the pesticide. Distribution was rapid throughout the plants with radioactivity detected in needles within an hour of root emersion. Distribution of acephate throughout the seedlings after 72 hours was predominantly in the needles. In another study using white spruce, Sundaram et al. (1977, in Lambert 1983) investigated systemic transport as a function of how Orthene is applied. They administered the insecticide by trunk implantation, basal bark painting, and foliar painting. Penetration was rapid; translocation within the plant was by xylem transport. Uptake and foliar accumulation through xylem transport was highest from trunk implantation, less in basal bark painting, and least in foliar painting.

Experiments with transport of acephate from spruce foliage treated with a simulated aerial spray application indicated that although there is rapid transport of the insecticide from root to leaf and/or needle, there is not a comparable transport of acephate from leaf and/or needle to other parts of the plant (Sundaram and Hopewell 1976, in Lambert 1983).

Richmond et al. (1978) report on the possible systemic action of acephate (Orthene 75 SP) as it moves with plant sugars into developing buds of Douglas-fir. Foliage protection was estimated to be effective for as long as 35 days. Acephate persisted at high levels for 10 days, then dropped over a 5-day period to lower levels for as long as 35 days. A Serbian study (Ostojic 1977) reported that acephate degraded rapidly on apples and grapes. The rate of degradation was highest during the first few days, and the author suggested 10 days as a safe interval to wait before harvest. In the United States, however, 21 days is the recommended waiting time.

3.1.2 Animals

Acephate is toxic to insects but only slightly toxic to vertebrates, being rapidly excreted in urine. Ando and Wakamatsu (1983) suggest that a part of acephate is metabolized to inactive substances in the liver of mammals. From 23 to 27 percent of acephate is metabolized in the body. In the body, acephate inhibits the ChE activity in plasma, red blood cells and the brain. In birds, the inhibition of brain ChE can be lethal (see sections 2.4 and 2.5.). Sublethal effects include: increased total liver lipids (triglycerides); decreased plasma triglycerides; changed activity in hepatic enzymes (i.e., reduced succinic dehydrogenase and increased lactic dehydrogenase). Choudhari and Chakratbarti (1984) believe there may be an acephate-induced impairment in the synthesis or release of the lipoprotein.

Ando and Wakamatsu (1983) report that acephate may combine with cytochrome oxidase and modify the configuration of the enzyme. The activity of the cytochrome oxidase was inhibited by acephate at alkaline pH but was insensitive to acephate at acid pH. The results of this research suggest that cytochrome oxidase and acephate may combine at alkaline pH.

Rojakovick and March (1972) found that acephate (Orthene) in insects was exceptionally poor as an in vitro inhibitor of AChE but is lethal in vivo, suggesting acephate may be converted enzymatically or chemically to a more potent biological inhibitor. Hussain and Oloffs (1980) reported that in vivo after 5 hours, 4 to 8 percent of Orthene in fish and 63 percent in larvae was converted to the more toxic Monitor by N-deacetylation following release of Orthene into a creek (maximum 1100 ppb in water). By 24 hours, however, both Orthene and Monitor were absent. No conversion to Monitor occurred in the water. Since update and conversion to Monitor occurred at highest rates in benthic larvae, there could be short-term effects for fish. However, since a spray program also increases the temporary drift of terrestrial insects into the water, the fish would be expected to take advantage of this food supply and thus avoid any adverse effect, even over an 8-hour period. Short-term sublethal effects can, however, occur in as little as 1 hour, as toxic concentrations of acephate are taken up by rainbow trout (Salmo gairdneri) in 1 hour or less (Klaverkamp and Hobden 1980). The concentration of acephate required to produce 50 percent inhibition of brain AChE is about 125 mmol/l. In vitro the incubation of acephate in fresh liver homogenate produced a greater increase in AChE inhibition, due to the activation by monenzymatic process or perhaps due to deactylation. Acephate is activated by metabolic process in fish.

In a field test with acephate, however, only suckers (Catostomus spp.), not salmonids (Salvelinus or Salmo), exhibited depressed AChE activity, (Rabeni and Stanley 1979). Suckers exhibited a 28 to 29 percent reduction in AChE and gradually returned to normal by day 8. The reason for this could be that the suckers are bottom feeders. Geen et al. (1981) reported that acephate levels in sediment increased to a maximum in 8 hours, as did the presence of the more toxic methamidophos; both acephate and methamidophos decreased by 96 hours. On the other hand, drift feeders, like salmonids were opportunistic feeders, and consumed the floating dead terrestrial insects that drifted into the stream following the spray. Very little uptake was directly from water. At 5 hours, acephate levels were reported as 11.3 to 42.0 ppb in fish compared to 987 to 1,141 ppb in water at sites 1 and 2 (Geen et al. 1981), representing a bioconcentration factor of about 0.01 to 0.036 from water to fish. This is very low and is supported by a low octanol/water partition coefficient for acephate of 0.0428 (Lambert 1983), also indicating low bioaccumulation potential. Hall and Kolbe (1980) further support this finding for acephate (98 percent purity) (5 ppm in water) in tadpoles (Rana catesbeiana), with no significant uptake and no significant change in mean ChE activity when tadpoles were fed to mallards. Thus, the addition of acephate to the environment would have only short-term nonlethal impacts on biota, and presents virtually no threat via the food chain.

Studies using food chain organisms in model ecosystems (Booth 1975, in Lambert 1983) containing algae, water flea, emergent plants, insects, and mosquito fish concluded that residues of acephate and its metabolites were not persistent. Acephate residues were found only in the model ecosystem water, and did not biomagnify along the food chain or accumulate in any ecosystem component. Metabolic fragments were found incorporated into various tissues, but no acephate residues were detected in fish tissues, the organism at the top of the food chain in the model system. Specifically, a marine diatom was grown for 1 week in solutions of acephate at concentrations of 1, 10, and 100 ppm. Neither acephate nor methamidophos bioconcentrated to any extent at any concentration.

Bluegills were continuously exposed to 0.01 and 1.0 mg/l acephate for 35 days. At 10 times, the concentration of acephate in water, residues in fish tissue occurred as long as exposure occurred. This uptake followed a dose-response relationship in which tissue levels were proportional to environmental levels of acephate in their water. Upon transfer to uncontaminated water, the acephate in the edible portions of the fish was reduced by more than 50 percent within 3 days. It was concluded that this level of bioaccumulation was minimal and posed no serious threat to the food chain (USDA 1976, in Lambert 1983).

Earthworms kept in soil treated with acephate at the equivalent of 2 lb a.i./acre demonstrated uptake of the insecticide at 0.125 to 0.5 percent of the soil concentration. Residues in the worms disappeared after they were transferred to untreated soil. No bioconcentration occurred (USDA 1976, in Lambert 1983).

Metabolic studies have been conducted on acephate in the rat, goat, quail, cow, hog, and chicken. The metabolism and fate of acephate in all species was essentially the same (Lambert 1983). Excretion is rapid and essentially complete. Most of the excretion took place in the first 12 hours after exposure, and only a low level of elimination was observed afterward. The main route of excretion is in the urine of mammals and the feces of birds. Most of the remaining dose was found in the breath (rat and quail), feces, and traces in the milk (cows and goats) and eggs (quail and chicken). Only traces of the applied dose were detected in the tissues during dosing, and these declined rapidly to below detection limits when dosing ceased. None of the body residues was concentrated in any one organ or tissue. There is no bioconcentration of acephate in the flesh, fat, or other tissues of mammals either during dosing or after cessation of dosing with acephate. Acephate does not bioconcentrate in the flesh, tissues (including fat), or eggs of birds (USDA 1976, in Lambert 1983).

3.2 Soil

Lambert (1983) reports the net degradation half-life in soil averages from 1 to 3 days. The half-life of acephate in soil depends on the soil type, moisture content, and initial acephate concentration. There is no photodegradation of acephate on soils (Chen 1987). The rate of breakdown, compared with other insecticides, is relatively rapid, ranging between 0.5 to 4 days in soils having moderate to low organic contents (Pack and Verrips 1988). Laboratory tests using 9 soil types dosed at 1.0 and 10.0 ppm levels, wet to field capacity, and aged under aerobic conditions, demonstrated the following half-life values (USDA 1976, in Lambert 1983):

		Half-life (days)	
Soil Origin	Soil Type	1.0 ppm	10 ppm
Clarkesburg, CA	Clay	1.5	1.5
Fresno, CA	Loam	1.3	3
Kettleman City, CA	Clay (high pH)	0.5	0.5
Ocoee, FL	Loamy sand	1	1
Mt. Holly, NJ	Sandy clay loam	0.5	1
Norwalk, IA	Silty clay loam	---	2
Greenville, MS	Clay (low pH)	---	1.5
Ocoee, FL	Muck	6	13
Riverside, CA	Loamy sand	---	4*

*20 ppm acephate added to soil.

Half-life in the high organic muck was significantly longer than in the other soils. Methamidophos, the primary metabolite of acephate, has half-lives in soils under similar conditions in the range of 2 to 6 days (Lambert 1983).

Degradation rates under actual field conditions may be slower than under laboratory conditions, but the rapid breakdown of acephate in soil compared with other insecticides follows a similar pattern. Lai (1987a, 1987c, 1987d, 1987e) reported on field condition dissipation rates for acephate and its metabolite methamidophos. The studies were performed on treated and untreated plots of approximately 2,500 ft^2. The following half-life values were reported:

Soil Origin	Soil Type	Half-life (days) Acephate	Half-life (days) Methamidophos
Cauliflower, FL	Sand (low pH)	1.95	3
Soybean, IA	Loam	1.96	*
Tobacco, MS	Silt loam	1.72	2
Bell Pepper, CA	Silt loam (high pH)	1.65	3

*Methamidophos levels too low to determine decay rate.

This situation means that there is no soil buildup of acephate or its metabolites from one season to the next (Lambert 1983).

The degradation of acephate and methamidophos in soil is due to microbiological action. In both clay and silty clay loam, the half-life of acephate was 1.5 days under nonsterile conditions, while sterile soil retained 80 to 95 percent of its initial dose. Four days after treatment, the sterile soil still contained 90 to 95 percent of its initial dose, while the nonsterile soil concentration was only 15 percent (Tucker 1972, in Lambert 1983). Under anaerobic conditions, lower degradation rates were measured and more of the degradation products were incorporated into the microorganisms than under aerobic conditions.

Laboratory studies using both column leaching and soil thin-layer techniques have shown that acephate and methamidophos are readily moved by water in soil with little or no retention by the soil particles. Pack (1987) reported soil adsorption coefficients (K_D) ranging from 0.12 to 0.68 for soils ranging from sandy clay loam to adobe clay. There is essentially no difference in the rate of leaching of acephate in soil that is wet to field capacity or in air-dried soil (Tucker 1972c). Moreover, there appears to be little effect on the leach rate of acephate from soil due to aging. In one aging test, soil samples were sprayed with acephate at 9 lb a.i./acre and allowed to age undisturbed except for watering in a greenhouse. After 21 weeks, the soil contained 0.005 ppm acephate or less, which was about 0.5 percent of the applied dose. The remaining residues

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were entirely leachable with no detectable bound acephate or methamidophos present in the soil (Tucker 1972b, in Lambert 1983).

A different behavior was observed for acephate and methamidophos degradation products. In one test, radiolabelled acephate was applied to soil at 2 ppm and incubated for 20 days. Approximately 15 percent of the total applied acephate remained in the soil after the incubation period. The soil was placed on top of columns of the same soil untreated and leached using 0.5 inches of water daily for 46 days. Only 0.3 percent of the applied radiocarbon was recovered in the leachates. Following leaching, the residual radioactivity was found to be almost entirely in the top three inches of soil (Lambert 1983).

The relatively high mobility of acephate in water systems raises the question of potential groundwater contamination. This was studied in a sandy Florida soil that represented an extreme case because of the porous nature of the soil and the amount of rainfall received by the test plots. Applications of acephate up to 3 times the recommended label rate were made. Maximum acephate levels were found 6 to 12 inches below the surface. None was found at or below 2.5 feet below the surface. The threat to groundwater appears to be negligible (Lambert 1983).

Only three studies are available on the fate of acephate in soils. Acephate appears to be degraded by microorganisms present in soil and does not pose any adverse effects upon soil microorganisms.

Focht and Joseph (1974) tested acephate (70 percent technical) and Monitor (20 ppm in soil) applied to a loamy sand, a silt loam, and a clay loam. Pesticides were applied on day 1, 15, and 36 and soils monitored for 50 days. Pesticides were applied at a rate 10 times greater than is normally used in the field (1.1 kg/ha), and no major effects were observed for actinomycetes, soil bacteria or fungi from either acephate or Monitor. No effect was demonstrated for ammonification, nitrification, sulfur oxidation, or respiration rates of these soil organisms.

Rosenberg and Alexander (1979) isolated two species of Pseudomonas from soils and sewage. Extracts of each of the two bacteria derived from organophosphate-grown cultures catalyzed the disappearance of Orthene. Orthene was metabolized by bacteria, and products of the enzyme breakdown were determined. The breakdown of the pesticide resulted from an induced enzyme or enzyme system, and the organism uses the organophosphate as a phosphorus source for growth.

Lindquist and Wolgamott (1980) found acephate used as a soil drench was most toxic to a mite and its predator. Rates used were extremely high, 150 to 1,200 ppm. Acephate spray remained toxic in soil for 3 weeks, and the soil drenches remained toxic to predators even longer. However, the authors could not separate the food-chain effects from residual toxicity. The authors concluded that acephate's usefulness could be limited by food-chain toxicity, especially if applied to the soil.

3.3 Water

Acephate is short-lived in water, especially in running water systems. It is soluble in water but fairly stable to hydrolysis. Rate of hydrolysis is not related to pH except at extreme pH values. Lambert (1983) reports the half-life for acephate for hydrolysis (days at 21 °C) as follows:

pH 3	65.5
pH 5	55.2
pH 7	46.7
pH 9	16.1

Geen et al. (1981) and Hussain and Oloffs (1980) reported the fate of Orthene released into a small stream at a rate of 1,100 ppb for 5 hours. Orthene residues remained high (800 to 1,200 ppb) for 5 hours, then decreased sharply. Within 96 hours, all traces had disappeared. No Monitor was found in water samples. The impact on the stream was limited and localized to within 2,000 m. Maximum concentration found within 8 hours, 2,000 m from release site, was 169 ppb. Only 2.7 ppb acephate was detected at this distance by 96 hours. Sediments, however, remove acephate from water and convert it to the more toxic methamidophos. Within 4 to 8 hours, methamidophos concentrations in sediment reach maximum (131.6 ppb) levels but by 96 hours were not detectable.

Rabeni and Stanley (1979) followed the effects of an aerial spray program of acephate on natural stream ecosystems. Residues remained for at least 2 days, disappearing at a rate of 40 percent per day due to chemical breakdown and flushing downstream. Acephate concentrations ranged from a high of 140 ppb 1 hour after the spray to 9 ppb 2 days later. The authors concluded that cautious use of acephate causes minimum ecological damage compared to organochlorine compounds.

Under field conditions, acephate and methamidophos dissipate more rapidly than was measured for buffered water systems. Ponds in Florida and Iowa were treated with 0.1 ppm acephate. Samples of water, bottom mud, and submerged vegetation were monitored. Acephate residues in water decreased with a half-life of 3 to 5 days. Half-lives ranged between 1 to 3 days in the bottom sediments. No methamidophos was detected, but this may have been due to the analytical techniques used (Lambert 1983).

3.4 Air

No literature is available on the fate of acephate in the atmosphere. Conditions should be avoided where drift could occur. Drift retardants are recommended for use with acephate aerial sprays.

Vapor pressure is 2×10^{-6} mm Hg at 25 °C and 1.7×10^{-6} mm Hg at 29 °C (Lambert 1983).

Lambert (1983) suggests that the relatively low vapor pressure of acephate implies that its expected concentration in air at ambient temperatures would be 0.02 mg/m^3 or 0.002 ppm. This would be a concentration in air in equilibrium with an exposed sample of pure material.

After application, acephate is not likely to co-distill from the plant surfaces with the transpiration water. This is likely due to the absorption of the active ingredients on and into the plant tissues.

Ambient air testing in rooms treated with 1 percent solution of acephate according to label instructions was conducted by Wright et al. (1981, in Lambert 1983) to determine the room concentrations after application. The highest ambient level of acephate detected was 2.9 μg/m^3 on the day after treatment, with rapid die-off to 0.3 μg/m^3 by the third day. This compares with the 20 μg/m^3 concentration anticipated on the basis of vapor pressure alone.

Lambert (1983) also reported for photolysis degradation. Light and dark experiments with acephate dissolved in water and deposited on glass and paper indicated that simulated sunlight did not affect the degree of degradation. It can be concluded that photolysis is not a significant factor in the persistence or degradation of acephate in the environment.

4.0 Exposure and Hazard Assessment

Forest workers, visitors, and resident or transient animals can be exposed to acephate from forest aerial applications. Animals and humans, can be exposed dermally, orally, or minimally by inhalation routes. Fish and aquatic insects can take up acephate from water and sediments and convert it to the more toxic methamidophos, which may be present for 96 hours, for example, following an accidental spill. The following discussion is based upon risk analyses performed by the USDA Forest Service (1984) in a Supplemental Draft Environmental Impact Statement on Gypsy Moth Suppression and Eradication Projects.

Acephate is reported to be noncarcinogenic (Seiler 1977, in USDA Forest Service 1985). No data are available on workers' exposure or cancer rates. Exposure studies are lacking for both workers and the general public. No data are available on animal exposure in the spray area. Epidemiological studies are lacking for cancer, birth defects, or systemic effects; therefore, the worst-case analysis was used for the EIS risk analysis. Worst-case doses range from 3 to 20 times above the no observed effects level (NOEL) for ChE inhibition are equivalent to the NOEL value for systemic effect in dogs but were 4 to 80 times below the NOEL for teratogenicity in dogs.

4.1 USDA Forest Service Application

The USDA Forest Service uses acephate as a spray from the ground as well as aerially. It also is applied as a systemic implant device or as granules. No data are available for spruce budworm control. For gypsy moths, the USDA Forest Service used acephate on 1 to 400 acres/year from 1979 to 1984 (Wolfe 1985). Typically workers involved in a spray program would be mixers/loaders and observers. For implants or pellets, only one worker would be in contact.

4.2 Exposure

Workers could be exposed dermally while mixing water soluble powdered acephate or could inhale dusts from the powder. Ford (1987) reports that 80 percent of total exposure for greenhouse workers would be to the hands. Spills and cleanup could result in higher short-term exposure. Observers in the field could be exposed by both dermal and inhalation routes at the time of a spray. Specific rates of exposure for workers are lacking for acephate. However, the worst-case doses are assumed for occupational exposure.

Accidental exposure due to truck or airplane spills also would result in worst-case doses. Truck spills present the greatest human health hazard, but have the lowest probability of occurrence. The USDA Forest

Service (1985) risk analysis model calculates 1 spill on land for every 93,000 vehicle trips or 1 spill into water for every 833,000 vehicle trips (realistic scenario). For the worst-case exposure, the probability of a spill is 1 spill on land for 50 million trips or 1 spill in water for 5,000 million trips. Worst-case doses are estimated to be 1 chance in 555. Some accident scenarios may have an impact on human health. Up to 35 people could be exposed to an accident involving a spill.

4.2.1 Occupational Dose

Ando and Wakamatsu (1982) report that the serum ChE activity of workers who were exposed to acephate was not affected when the concentration of acephate in serum was less than 200 ppm. Occupational exposure at a manufacture facility resulted in workers having 5 ppm acephate in urine. No adverse effects nor inhibition of blood ChE activity was noted (see section 2.5.3). It is possible that realistic doses over a long period of time could cause measurable and temporary depression of ChE activity. Prolonged exposure to worst-case exposure rates would probably cause some systemic effects and measurable ChE inhibition. The USDA Forest Service (1985) estimates the probability of a worst-case dose is 0.0018. The methodology, however, does not account for the 25 percent of acephate which is metabolized, and therefore, even the realistic dose is overestimated. The worst-case exposure scenario for occupational exposure exceeds the allowable daily intake (ADI) set by the Environmental Protection Agency.

Occupational doses are shown on table 4-1 and are compared to environmental exposures. Inhalation doses are in dermal doses. The total realistic expected dose to mixers/loaders is 0.035 mg/kg/day, which is above the ADI. The worst-case dose for observers is 0.044 mg/kg/day which is above the ADI (USDA Forest Service 1985). Realistic doses to observers are within the ADI. Merricks (1987) reported on mixers/loaders and applicators using acephate on a Florida golf course. All workers used Tyvex coveralls and latex gloves. Tests showed that there was little dermal exposure on protected workers.

Schneeberger (1985) calculated the weighted lifetime risk of cancer for acephate as 2.4 (suppression) plus 1.4 (eradication) or a cumulative risk of cancer of 3.8 chances in a million. However, the USDA Forest Service states that acephate is not carcinogenic (Seiler 1977, in USDA Forest Service 1985).

4.2.2 Environmental Exposure

Environmental exposure to humans occurs when forest visitors or others not directly involved in spray operations come in contact with spray or sprayed foliage, inhale spray mist, eat plants or animals that are

Table 4-1

Summary of Occupational Dose, Environmental Exposure and Toxicity for Acephate

	Exposure (mg/kg/day)[a]	
	Dermal/Inhalation[b]	Oral
Occupational Dose		
Mixer/loader	0.035	No dose calculated
Observer	0.0017	No dose calculated
Environmental Exposure		
Fish[c]	0.05	0.05
Large animal (goat)	0.23	0.29
Small animal (rabbit)	0.26	0.30
Humans		
Direct exposure	0.0017	NA
Near drift	0.0011	
Ingestion		
Fish	NA	0.0004
Rabbit	NA	0.0000001
Goat	NA	0.000002
Water	NA	0.0014
Vegetables and Fruits	NA	0.007
Toxicity Summary	LD_{50} = 2,000 to 10,250 mg/kg slightly toxic.	LD_{50} = 233 to 1,800 mg/kg slightly toxic to mammals: subchronic may inhibit ChE acitivity. NOEL = 2.5 to 0.75 mg/kg
	Most studies LC_{50} greater >100 mg/l, practically nontoxic for fish.	

[a] Assumes aerial application fine droplets 1 lb a.i./acre acephate (U.S. Forest Service 1985) realistic doses given.

[b] Included with dermal exposure, inhalation only 0.2 percent of exposure.

[c] Fish exposure deliberate overestimate from immersion, exposure to about 50 ppb.

NA = Nonapplicable data.

contaminated, or drink water containing acephate. Animals, both terrestrial and aquatic, are subject to environmental exposures as well.

4.2.2.1 Dermal Exposures. Human dermal environmental exposures would be less than occupational exposures, since only spray operators and observers are directly involved with actual activities on the spray units. A casual visitor to spray units should be expected to receive an exposure much less by orders of magnitude than that of the mixer/loader or backpack sprayer. Animals in the target spray zone, however, are subject to dermal exposure from aerial application of acephate. It was assumed that animals would not be sprayed directly by backpack applicators, and that the animals ventral surfaces would contact foliage that had been sprayed with liquid formulations. Thus, approximately half of an animal's body surface would contact sprayed vegetation, resulting in an exposure level equal to half of the exposure that would be calculated for aerial application methods at the maximum rate of application (aerial application amounts for exposure of the dorsal surface as well as ventral). Rabbits and goats, representing small and large game animals, respectively, have the following estimated dermal exposures (USDA Forest Service 1985).

Dermal exposures and doses for goats assume the following:

- The goat weighs 40 kg (88 lbs) and has 2.3 m^2 surface area.
- The dorsal surface is contacted by deposited spray.
- Treated foliage contacts the goat's sides and stomach as the animal walks through it, at a concentration equivalent to direct deposition.
- Dermal absorption rate ranges from 1 to 10 percent. A 10 percent absorption rate is considered to be worst-case assumption because dermal toxicity studies are conducted on shaved animals. Animal hair acts like human clothing, protecting against dermal exposure.

The estimated dermal exposure to a goat is 2.02 mg/kg (35 mg/m^2 x 2.3 m^2/goat x goat/40 kg) for each 1 lb a.i. applied per acre. The estimated dermal dose to the goat ranges from 0.020 to 0.202 mg/kg (2.02 mg/kg x 0.01 or 0.1) depending on the dermal absorption rate.

Dermal exposures and doses for rabbits assume the following:

- The rabbit weighs 2.0 kg and has 0.23 m^2 surface area.
- The dorsal surface is contacted by deposited spray.

- The ventral surface is contacted by treated foliage as the animal walks through it, with an insecticide concentration equivalent to direct deposition.

- Dermal absorption rate ranges from 1 to 10 percent, with the latter rate considered a worst-case assumption for reasons previously stated.

The estimate dermal exposure to rabbits is 4.03 mg/kg (35 mg/m^2 x 0.23 m^2 rabbit x rabbit/2 kg) for each 1 lb a.i. applied per acre. The estimated dermal dose to rabbits ranges from 0.040 to 0.403 mg/kg (4.03 mg/kg x 0.01 or 0.1).

Individuals in treatment blocks, or persons within the drift of the application site, may be exposed to insecticides by dermal contact, inhalation, or ingestion. However, as seen by the data presented previously, dermal and inhalation exposures to persons downwind are low relative to those experienced by project personnel. For example, residents or casual visitors to spray blocks would not be expected to receive a dermal exposure higher than project personnel who are in the treatment area and receiving direct spray deposition. Consequently, occupational and resident exposures are estimated by the USDA Forest Service (1985) as follows:

Dermal and Inhalation Doses for Humans (mg/kg/day)*

	Realistic	Worst Case
Workers		
- Mixers/loaders	0.042	0.1
- Observers	0.002	0.029
Residents		
- Direct exposure	0.002	0.004
- Near range drift	0.0013	0.0023
- Indirect exposure	0.0004	0.0009

4.2.2.2 Inhalation Exposures. Human inhalation environmental exposures would be less than occupational exposures since spray operators, involved with activities on the spray units are more likely to be subject to spray mist than are casual visitors. Thus, a casual forest visitor should be expected to receive an inhalation exposure that is orders of magnitude less than that of a backpack sprayer. Environmental inhalation exposures of animals in the spray target area would occur on a one-time basis and would be limited to a time period that can be measured in minutes following application of liquid formulations. Inhalation of acephate following its application would be negligible. Inhalation exposure is therefore expected to be so small that it can be included with dermal exposure.

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*Inhalation dose is only 0.2 percent of exposure and is therefore included with dermal exposure.

Table 4-1 shows the total expected (realistic) doses due to occupational and environmental exposure. These doses are compared to toxicity data from mammals and fish. In some cases, the USDA Forest Service model predicted worst-case doses that exceed EPA standards, and these are identified in the text where appropriate.

Exposure of residents who live adjacent to or near treatment areas can result from drift. Witt (1984) reviewed a number of drift studies (which referred to 75 to 100 μm drop size) and reported drift values of 2, 0.5, and 0.15 percent of the amount deposited on targets at distances from the application site of 1/4, 1/2, and 1 mile, respectively. These values are used for calculating doses received from insecticidal drift because they are based on actual drift data, and involve droplet sizes typical of gypsy moth spray programs. Extrapolation of this data for near range drift (250 feet from the treatment area) shows the dose to be approximately 67 percent of that for a resident in the treatment area. For the USDA analysis then, the realistic and worst-case doses for near range drift is calculated to be 0.0013 mg/kg (0.002 x 0.67), and 0.0023 mg/kg (0.004 x 0.67), respectively (USDA Forest Service 1985).

4.2.2.3 Immersion Exposures. Aquatic organisms are subject to environmental exposures to acephate from immersion in streams and ponds adjacent to target spray zones. Environmental exposures for aquatic organisms are expected to be negligible, about 0.05 mg/kg/day, which is an overestimate (USDA Forest Service 1985). Acephate is not very toxic to fish (LD_{50} about 2,800 ppm) (Hussain and Oloffs 1980). Rabeni and Stanley (1979) report levels of acephate in water following a spray by helicopter. One hour after spray water contained 113 to 140 ppb acephate, after 1 day 13 to 65 ppb, and after 2 days 9 to 41 ppb. Thus, the exposure rate of 0.05 mg/kg (ppm) is typical of exposures within 24 hours of a spray, is well below the lethal dose and did not cause significant reduction in acetylcholinesterase activity in fish brains.

4.2.2.4 Oral Exposures. Oral environmental exposure could result from wildlife eating sprayed foliage or from human consumption of large or small animals or fish. Both humans an animals could drink contaminated water, which could present a significant risk, for example, within a day or two of a spill of acephate into open water.

Oral exposures and doses for goats and rabbits assume the following:

- Post spray concentration insecticides in browse and food foliage ranges from 10 ppm (realistic) to 100 ppm (worst case).

- A typical goat weighs 40 kg (88 lbs) and eats 1.15 kg (2.5 lbs) field weight foliage per day. A typical rabbit weights 2.0 kg, eats 60 g of food (dry weight) daily, and receives a dose of 0.03 mg/kg/day for each ppm in food (USDA Forest Service 1985).

- Oral dose equals oral exposure.

Thus, the realistic and worst-case exposures for a goat due to ingestion would be 0.29 mg/kg/day (10 mg/kg x 1.15 kg/goat/day x goat/40 kg) and 2.88 mg/kg/day (100 mg/kg x 1.15 kg/goat/day x goat/40 kg) for each 1 lb a.i./acre applied. Estimated doses are the same: 0.29 and 2.88 mg/kg/day, respectively (USDA Forest Service 1985).

Similarly, a typical rabbit would have realistic and worst-case exposures of 0.3 mg/kg/day (10 ppm x 0.03 mg/kg/day/ppm) and 3.0 mg/kg/day (100 ppm x 0.03 mg/kg/day/ppm) for each 1 lb a.i./acre applied. Estimated doses are the same: 0.03 and 3.0 mg/kg/day, respectively (USDA Forest Service 1985).

The estimated dose to humans for drinking contaminated water and eating contaminated foods is equal to 0.0088 mg/kg/day, for worst-case and 0.0071 mg/kg/day for a realistic dose. These levels are well within the EPA established ADI and NOEL thresholds for the realistic dose, but are higher than acceptable ADI and NOEL for the worst-case dose. When diet is combined with occupational dose or with dermal exposure (direct or indirect), then that dose exceeds the ADI and NOEL threshold levels. The chance of a daily consumption of contaminated food (for worst-case dose) has a probability of occurrence of 1.8×10^{-3} or 1 chance in 500 (USDA Forest Service 1985). It is not known whether this value is outside the acceptable margin of safety, which is 10 for acephate.

Consumption of water into which aircraft spills have occurred would result in a dose (worst case) that slightly exceeds the ADI.

4.3 Hazard Assessment

Like other organophosphates, acephate is not very persistent in the environment. It has a short half-life in soil and is biodegraded by microorganisms. It is soluble in water with a high soil mobility but would not be expected to leach into groundwater. It has a moderate persistence in natural waters, is subject to hydrolysis, and lasts only a few days following a spray. It has a moderate systemic persistence in plants and may remain at toxic levels for 3 weeks. Invertebrates convert the acephate to the more toxic methamidophos within a short time, but there appears to be no bioaccumulation in food-chain organisms (Lambert 1983). The relatively low vapor pressure of acephate would result in very low concentrations in air.

While acephate is very toxic to pest species, it is only moderately to slightly toxic to mammals. Mammals metabolize acephate; excretion is rapid and essentially complete (Lambert 1983). Acephate was nearly 50 times less toxic to mammals than its metabolite, methamidophos. In birds and fish, acephate may cause a temporary depression in brain ChE, but no mortalities have been observed in the field following acephate sprays. Acephate is, however, toxic to nontarget species such as honey bees.

Acephate is noncarcinogenic, but teratogenic and mutagenic effects have been reported in the literature. Schneeberger (1985) calculated the cumulative chances as a weighted lifetime risk to cancer from acephate as 3.8×10^{-6}, within the "acephate risk" level of federal regulation, but more of a risk than carbaryl, diflubenzuron or trichlorfon, all less than 1×10^{-6} risk.

In USDA Forest Service (1985) risk analysis, the realistic dose calculated was hundreds to thousands of times below the teratogenic NOEL. Even worst-case doses for workers were 100 times lower than the teratogenic NOEL by an acephate margin of safety.

Realistic doses, however, to occupational exposure exceed the ADI. For worst-case doses, the ADI would be exceeded for dietary exposure, for all occupational exposure, and when diet is combined with other direct and indirect dermal exposures. One worst-case dose is estimated for every 555 realistic doses. Inhalation exposure is insignificant.

The most severe human impacts would result from dermal exposures as a result of truck or aerial spills. Aircraft spills are 47 to 177 times below the highest exposure levels tested with no effects. Up to 35 people could be exposed to such an accident, but fatalities are unlikely. Symptoms of ChE inhibition are possible. A worst-case dose from drinking water following an aircraft spill results in a dose slightly exceeding the ADI.

Truck spills could result in exposures up to twice as much as the highest exposure levels tested with no effects for dermal exposure. Spills in water could result in doses exceeding the ADI by 10 to 18 times. The results would be ChE inhibition and symptoms of that effect.

The USDA Forest Service (1985) showed both realistic and worst-case doses that are within an acceptable margin of safety. Accidents are an exception. Some accidents could have a negative human health impact.

Appendix A

Summary of Acephate Toxicity Data for Microorganisms

Appendix A

Summary of Acephate Toxicity Data for Microorganisms

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Acephate (94% technical)	Aquatic bacterium *Chromobacterium lividum*; (8 bacteria isolates) *Pseudomonas fluorescens*	1 mg/ml to culture flasks in triplicate to equal 1 ppm acetate and 1,000 ppm acetate to lake water	48-hr culture acephate added 15 to 30 min. after innoculation, culture harvested for 10 min. at 4 °C, doubling times 9 hrs	Increased doubling time, decrease maximum cellular yield, reduced cell size, (1,000 ppm flask); total glucose and amino acid uptake rate reduced (1,000 ppm); no effect on cycloleucine uptake.	NA	Williams and Albright 1983
Acephate (70% technical)	Actinomycetes, bacteria, soil fungi	20 µ/g soil plate and incubate monitor	50 days	No adverse effect upon soil microorganisms; nor any effect on ammonification, nitrification, sulfur oxidation, or respiration rate.	NA	Pocht and Joseph 1974

NA = data not available.

Appendix B

Summary of Acephate Toxicity Data for Invertebrates

Appendix B

Summary of Acephate Toxicity Data for Invertebrates

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Acephate (Orthene 94%)	Scud (Gammarus pseudolimnaeus)	Static bioassay	96 hrs	LC_{50} >100 ppm	Tested at 18 °C.	Schoettger and Mauck 1978
Analogs of Orthene (O, S, & N)	None given	None given	None given	Best analogs found to combine high insecticidal activity with low mammal toxicity.	None	Magee 1973
Acephate	Red flour beetle (Tribolium castaneum)	Topical treatment, 100 insects	None given	Acephate toxicity high: LD_{50} = 0.138 to 0.163 mg/g of insect weight. LD_{95} = 0.551 to 0.894.	Malathion-resistant beetles.	Zettler and Jones 1977
Acephate (Orthene 75 S)	Aquatic invertebrates	Immersion 1,000 ppm into a natural stream; 10 trout and 50 insects/cage	5 hrs	No mortality in fish or insects; animals and sediments declined to trace in 24 hrs and to trace in water in 96 hrs. Impacts limited and localized.	--	
Acephate (94% technical)	G. pseudolimnaeus	Immersion buffered water bioassay	96 hrs	LC_{50} = >50 mg/l	--	Johnson and Finley 1980
	Stonefly naid Pteronarcella		96 hrs	LC_{50} = 0.5 mg/l	Increase pH to 8.5 caused increase in LC_{50}.	
	Skwala	Immersion buffered water bioassay	96 hrs	LC_{50} = 12 mg/l		
	Chironomous	Immersion buffered water bioassay	96 hrs	LC_{50} = >1,000 mg/l	--	
Acephate (75% soluble)	Skwala	Immersion buffered water bioassay	96 hrs	LC_{50} = 12 mg/l	--	Johnson and Finley 1980

Appendix B (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Acephate (Orthene)	Honey bees *Apis mellifera*	Dermal spray 748 g/ha	--	Most at the colonies that survived the sprayings lived through the winter (94%); queens laid well, no permanent damage.	Test was for mitigation measures.	Moffett et al. 1979
(Orthene + paratox)	Honey bees *Apis mellifera*	Dermal spray 1.1231 g + 2.34/ha	--			
Orthene and Monitor	House fly Diptera: Muscidae	LD_{25}, LD_{50}, LD_{75} µ/droplets topically on abdomen	24 hrs	Decrease *in vivo* AChE activity in 12 hrs, exceptionally poor *in vitro* inhibitor of AChE, but lethal *in vivo* inhibition.	May be converted by enzymes or chemically *in vivo* to a biologically more potent inhibition.	Rojakovick and March 1972
Orthene and Monitor	Stream invertebrates	Aerial spray natural stream	4 days	Aquatic insect larva showed highest uptake of Orthene and highest conversion to Monitor, but levels dropped sharply to near 0 in 8 hrs; no mortality observed.	Orthene persistent in water.	Hussain and Oloffs 1980
Acephate	Tobacco budworm (*Heliothis virescens*)	Spray, topical application 21 l/ha, 2 to 5 times in July	--	LD_{50} - 0.041 mg/g. 1.12 kg/ha significantly reduced boll damage.	--	Wolfenbarger and Harding 1980
Acephate	*Myzus persicae*	Spray on peaches 500 ppm	4 days 22 days 51 days	0.3% living 0% living 96% living	Italian study	Mowlam et al. 1977
Acephate (Orthene medicaps)	Spruce budworm (*Choristoneura occidentalis*)	1 cartridge 0.8 g a.i./10 cm of tree trunk	28 days	Significant reduction in larvae/100 buds (<10 larvae/100 buds) LC_{50} - 14 to 18 ppm (fed).	--	Reardon and Haskett 1981
Acephate (Orthene 75 SP)	Nettle caterpillars (*Setora nitens*) (*Thosea asigna*)	Trunk injection 6.0 g a.i./tree	5 days	402 caterpillars survived	Poor performance; three applications required	Hutauruk and Sipayung 1978

Appendix B (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Acephate (Orthene medicaps)	Spruce budworm (C. occidentalis	2 consecutive yearly applications; systemic implants 0.8 g a.i.	Annual	Significantly fewer budworm larvae/100 buds.	--	Reardon 1984
Acephate 75 S and medicaps	Pine needleminer (Coleotechnites milleri)	Spray and trunk implants 1.25 g a.i./implant; acephate 454 g a.i./378 l water	4 wks	Spray very effective (99.2%) against larvae implants - solution 94.1% effective - medicap 49% effective	Tree implant residue longer lasting (4 weeks).	Brown et al. 1979
Acephate 75 SP (Orthene)	Leafminer (Liriomyza sativae)	Greenhouse tomatoes; spray 0.015, 0.03, 0.06, 0.09, and 0.12% a.i./plant	21 days	0.015 less effective than higher conc.; leafminer control 84 to 94% effective.	Higher rates leave residue up to 57 ppm after 3 wks.	Lindquist and Krueger 1975
Acephate and methamidophos	Citrus blackfly (Aleurocanthus woglumi)	0.6 g/l hydraulic spray twice at 21 day interval; 1.6 g/l third application with portable mist-blower.	5 mos	Significant conversion of acephate to methamidophos; residue persistent 3 months; 1/2 life on foliage 8 + days, rapid disappearance reduces risk.	--	Fitzpatrick and Dogan 1980
Acephate	Invertebrate stream drift and benthos	Aerial spray	8 days	Standing crop unchanged; increased drift benthos. Invertebrate community disrupted; minor short-term effects.	No species given; poor quantification.	Rebeni and Stanley 1979
Acephate Orthene	Mysid shrimp (Mysidopsis bahia)	Acute flow-through immersion marine water.	96 hrs	LC_{50} = 7,300 $\mu g/l$	--	U.S. Environmental Protection Agency 1981
Ortho 12,420 (1.9 technical/l)	Mysid shrimp (Mysidopsis bahia)	Life cycle in marine water chronic (1,300 to 10,000 $\mu g/l$	28 days	Adverse effects on reproduction by first and second week.	Maximum acceptable toxicant concentration >580 $\mu g/l$, >1,400 $\mu g/l$.	U.S. Environmental Protection Agency 1981

Appendix B (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Acephate (Orthene)	Pink shrimp (Penaeus duorarum)	Acute flow-through immersion	96 hrs	LC_{50} = 3,800 μg/l	Acute toxicity lowest test.	U.S. Environmental Protection Agency 1981
Acephate (Orthene)	Pink shrimp (Penaeus duorarum)	Acute static immersion	96 hrs	LC_{50} = >10,000 μg/l	Nontarget	U.S. Environmental Protection Agency 1981
Acephate (Orthene)	Embryo oysters (Crassostrea virginica)	Acute static immersion	48 hrs	EC_{50} = 150,000 μg/l	Nontarget	U.S. Environmental Protection Agency 1981
Acephate (140 and 105)	14 species of grasshoppers (Orthoptera: Acrididae)	Aerial spray 105 g a.i./ha	4 days 10 days	80 to 94% mortality 87 to 97% mortality	Target species	Foster et al. 1983
Acephate	Lygus hesperus	Topical application	24 hrs	LD_{50} = 0.13 to 4.16 μg/specimen	Target species	Leigh et al. 1977
Acephate (0.1 to 0.5%)	Cutworm larvae (Peridroma saucia)	Oral	24 hrs 48 hrs	60 to 90% mortality on peppermint 100% mortality on snap beans	Target species	Berry et al. 1980
Acephate (5%)	Harlequin bug (Murgantia histrionica)	Spray 40 gal of water/acre 2 lb a.i./acre 1.5 lb a.i./acre 1.0 lb a.i./acre 0.73 lb a.i./acre	3 days	 100% adult reduction 99.8% adult reduction 99.8% adult reduction 99.4% adult reduction	Target species	Rogers and Howell 1973
Acephate	Mite (Phytoseiulus persimilis) and (Tetranychus urticae)	Oral on sprayed plants. 75, 150, 300, 600, 1,200 ppm fed 1 to 3 days, monitored 3 to 5 days	24 to 72 hrs	Toxic 3 wks after application (600-1,200 ppm) drenches more toxic than foliar spray. Mortality after 48 hrs: 75 ppm = 20 to 44%, 150 ppm = 30 to 42%; 300 ppm = 12 to 60%. Significantly toxicity via food chain.	--	Lindquist and Wolgamott 1980

Appendix B (Concluded)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Orthene	Green rice leaf-hopper (Nephotettix cincticeps)	Not reported	NA	LC_{50} 12.6 ppm male LC_{50} 7.1 ppm female	Target species	Lambert 1983
Orthene	Western spruce budworm (Choristoneura occidentalis)	Oral	NA	LC_{50} 7.7 ppm	6th stage larvae	Lambert 1983
Orthene	Spruce budworm (Choristoneura fumiferana)	Topical	NA	LD_{50} 32.6 μg/g body weight	Target species	Lambert 1983
	(C. occidentalis)			LD_{50} 21.6 to 55.7 μg/g body weight	Target species	Lambert 1983
	(C. viridus)			LD_{50} 45.3 μg/g body weight	Target species	Lambert 1983
Orthene	Potato tuberworm (Phthorimaea operculetta)	Topical	NA	LD_{50}'s 126 to 1,770 μg/g body weight	Target species	Lambert 1983
Orthene	Green lacewing (Chrysopa carnea)	Topical	NA	LD_{50} 106 μg/g body weight	Larvae	Lambert 1983

NA - data not available; AChE - acetylcholinesterase.

Appendix C

Summary of Acephate Toxicity Data for Fish

Appendix C

Summary of Acephate Toxicity Data for Fish

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Acephate	Rainbow trout (*Salmo gairdneri*)	Intermittent flow-through bioassay	24 hrs	LC_{50}'s for two sets of tests were 1,050 and 895 ppm. Median survival times ranged from 8.5 hrs at 1,625 ppm to 21.4 hrs at 950 ppm.	Temp. 15 °C, pH 6.81 to 6.92, continuous aeration. Inhibiting effect of acephate on brain ChE was reported to be low.	Klaverkamp et al. 1975
Acephate (Orthene; commerical-75.5% and technical-97%, grade)	Cypriniform (*Mystus vittatus*)	Static bioassay with 24-hr renewal	Up to 96 hrs	24-hr LC_{50}'s were 159.4 and 1,705 ppm for commercial and technical formulations. 48-hr LC_{50}'s were 157.5 and 1,675 ppm for commercial and technical formulations. 96-hr LC_{50}'s were 155.4 and 1,592 ppm for commercial and technical formulations	Toxic effects on behavior resulted in rapid, jerky swimming, difficulty in respiration, and opercular movement. Water temp. was 21.4 ± 2 °C, pH 7.4 to 7.5.	Verma et al. 1981
Acephate (Orthene; technical grade, 94%)	Brook trout	Acute, assumed to be static bioassay	96 hrs	LC_{50} >100 ppm	Water temp. 7 and 12 °C, pH 6.5 to 9.0, hardness 40 and and 320 ppm.	Schoettger and Mauck 1978
Acephate	Rainbow trout (*Salmo gairdneri*)	*In vitro*	NA	Carbonic anhydrase activity in blood and gills significantly inhibited ($p <0.05$) at acephate conc. above 100 mg/l. Conc. of 38,000 mg/l and 8,900 mg/l produced 50% reduction of carbonic anhydrase activity in gill and in red blood cells, respectively.	--	Watson et al. 1982

Appendix C (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Acephate	Rainbow trout (Salmo gairdneri) fingerlings and adults	Immersion 850 to 1,000 mg/l	96 hrs	Temp. stress pronounced within 24 hrs; decrease in heart rate, increase in ventilation rate and amplitude in adults; ChE inhibition in gills, heart and serum tissue.	No LD_{50} data	Duangsawasdi 1977
Acephate (99.31%)	Rainbow trout (Salmo gairdneri)	Bioassay flow-through	40 mins incubation	LD_{50} about 125 m mol/l; 6.3% ChE inhibition before, 9.5-14.7% after incubation.	Incubation caused greater ChE inhibition.	Klaverkamp and Hobdeñ 1980
Acephate (94% technical)	Cutthroat trout	Immersion buffered water bioassay 6 conc.	96 hrs	LC_{50} = >100 mg/l	Alteration of pH, temp., or hardness did not alter toxicity.	Johnson and Finley 1980
	Rainbow trout	Immersion buffered water bioassay 6 conc.	96 hrs	LC_{50} = 1,100 mg/l	Alteration of pH, temp., or hardness did not alter toxicity.	Johnson and Finley 1980
	Brook trout	Immersion buffered water bioassay 6 conc.	96 hrs	LC_{50} = >100 mg/l	Alteration of pH, temp., or hardness did not alter toxicity.	Johnson and Finley 1980
	Fathead minnow	Immersion buffered water bioassay 6 conc.	96 hrs	LC_{50} = >1,000 mg/l	Alteration of pH, temp., or hardness did not alter toxicity.	Johnson and Finley 1980
	Channel catfish	Immersion buffered water bioassay 6 conc.	96 hrs	LC_{50} = >1,000 mg/l	Alteration of pH, temp., or hardness did not alter toxicity.	Johnson and Finley 1980
	Bluegill	Immersion buffered water bioassay 6 conc.	96 hrs	LC_{50} = >1,000 mg/l	Alteration of pH, temp., or hardness did not alter toxicity.	Johnson and Finley 1980

Appendix C (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Acephate (94% technical)	Yellow perch	Immersion buffered water bioassay 6 conc.	96 hrs	LC_{50} = >50 mg/l	Alteration of pH, temp., or hardness did not alter toxicity.	Johnson and Finley 1980
Acephate (75% soluble powder)	Cutthroat trout	Immersion buffered water bioassay 6 conc.	96 hrs	LC_{50} = >100 mg/l	Alteration of pH, temp., or hardness did not alter toxicity.	Johnson and Finley 1980
	Rainbow trout	Immersion buffered water bioassay 6 conc.	96 hrs	LC_{50} = 730 mg/l	Alteration of pH, temp., or hardness did not alter toxicity.	Johnson and Finley 1980
	Fathead minnow	Immersion buffered water bioassay 6 conc.	96 hrs	LC_{50} = >1,000 mg/l	Alteration of pH, temp., or hardness did not alter toxicity.	Johnson and Finley 1980
	Channel catfish	Immersion buffered water bioassay 6 conc.	96 hrs	LC_{50} = 560 to 1,000 mg/l	Alteration of pH, temp., or hardness did not alter toxicity.	Johnson and Finley 1980
	Bluegill	Immersion buffered water bioassay 6 conc.	96 hrs	LC_{50} = >1,000 mg/l	Alteration of pH, temp., or hardness did not alter toxicity.	Johnson and Finley 1980
	Yellow perch	Immersion buffered water bioassay 6 conc.	96 hrs	LC_{50} = >100 mg/l	Alteration of pH, temp., or hardness did not alter toxicity.	Johnson and Finley 1980

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Acephate	Brook trout (Salvelinus fontinalis); Landlocked salmon (Salmo salar); Longnose sucker (Catostomos catostomos); Common sucker (C. commersoni)	Aerial spray	3 hrs (water) 5 mos (fish)	Minor, short-term perturbation in stream ecosystem; brain ChE reduced in suckers; others altered diet but growth unaffected.	Acephate in water 1/10,000 of toxic level; 96 hrs.	Rabeni and Stanley 1979
Acephate (1.9 technical/l)	Sheepshead minnow (Cyprinodon variegatus)	Acute static marine	96 hrs	LC_{50} = >3,200 μg/l	Chronic exposure intoxicates fish.	U.S. Environmental Protection Agency 1981
	Spot (Leiostomus xanthurus)	Acute static marine	96 hrs	LC_{50} = >10,000 μg/l	Loose equilibrium	
	Pinfish (Lagodon rhomboides)	Acute flow-through	96 hrs	LC_{50} = >85,000 μg/l		
Orthene and Monitor	Rainbow trout	10/cage in natural stream following spray	4 days	Fish convert Orthene (4-8%) to Monitor in tissue; no mortality observed; uptake of fish from water very low (1-4%), but 1 day tissues were cleared.	Conc. in stream decreased to near 0 in 4 days.	Hussain and Oloffs 1980
Acephate (94% technical)	Cutthroat trout	100 mg/l immersion	96 hrs	No LC_{50} given	--	Lambert 1983
Orthene 75 S	Largemouth bass	1,725 mg/l immersion	96 hrs	No LC_{50} given	--	Lambert 1983
Orthene 75 S	Channel catfish	2,230 mg/l immersion	96 hrs	No LC_{50} given	--	Lambert 1983
Orthene 75 S	Mosquito fish	6,650 mg/l immersion	96 hrs	No LC_{50} given	--	Lambert 1983
Acephate	Goldfish	9,500 mg/l immersion	96 hrs	No LC_{50} given	--	Lambert 1983

NA - data not available; ChE - cholinesterase.

Appendix D

Summary of Acephate Toxicity Data for Birds

Appendix D

Summary of Acephate Toxicity Data for Birds

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Acephate (Orthene insect spray)	Mallard (eggs)	Eggs immersed in aqueous solution.	Eggs immersed 30 sec on day 3 of development and observed to day 18	LC_{50} equivalent to application at 54 lb/acre (95% confidence limits of 41 to 71 lb/acre).	Reduced growth and abnormal survivors were observed at doses less than and greater than the LC_{50} value. Abnormalities included malformation of bill, eye, and neck; edema, and stunting.	Hoffman and Albers 1984
Acephate	Quail	Inhalation of respirable aerosol	Up to 5 hrs	Animals were exposed to aerosol conc. of about 2.2 mg/l. Three of 6 quails died when exposed for 100 min.	--	Berteau and Chiles 1978
Acephate	Mallard (ducklings)	Oral, single dose	Observed for 16 hrs after dosing	Six ducklings each were fed 25, 75, 125, 225, or 350 mg acephate/kg. One of 6 fed 225 mg/kg died, all others survived.	Treated ducklings showed decreased brain and plasma ChE activity. Brain ChE activity was 78, 74, 55, 60, and 48% of controls and plasma ChE activity was 50, 43, 37, 26, and 36% of controls in animals fed 25, 75, 125, 225, and 350 mg/kg, respectively. At 350 mg/kg, an estimated 5 days and 24 days were required for 80 and 100% brain ChE recovery, respectively.	Fleming and Bradbury 1981

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Acephate (Orthene 75 S)	Dark-eyed junco	Acute oral (gastric lavage)	Single dose followed by 5 hrs of observation	Birds given 37.5, 75, 106, 212, or 300 mg acephate/kg body weight. Deaths occurred within 75 min. to 5 hrs. Calculated LD_{50} was 106 mg/kg.	Signs of toxicity included fluffed feathers, depression, dyspnea, ataxia, wing drop, falling stiffly with wings spread, tremors, sternal or lateral prostration, and convulsions. Brain ChE depression was 66% below controls in low dose and 91.4% below controls in high dose group. Residue acephate brain conc. of 2 mg/kg were reported in all birds that died.	Zinkl et al. 1981
Acephate (Orthene 75 S)	Dark-eyed junco	Subchronic oral	Birds fed acephate in diet 5 days as mg/kg feed	Birds fed 625, 884, 1,240, 1,780, 2,500, 3,536, or 5,000 mg/kg. Calculated LC_{50} was 1,495 mg/kg (ppm).	Signs of toxicity included ataxia, depression, lassitude, and emaciation. Minimum brain ChE inhibition in a that died was 38.3%. No birds had brain acephate residues greater than 2 mg/kg (ppm). Data suggests metabolism of acephate to methamidophos.	Zinkl et al. 1981

Appendix D (Concluded)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Acephate (Orthene 75 S)	Western tanager, Dark-eyed junco, Swainson's thrush	0.57 kg/ha aerial spray, dermal exposure	3 to 6 hrs after spray, and 1, 3, 6, 25, and 26 days after treatment	Day 1, 50% of birds had depressed brain ChE activity; day 2, 54% depressed, day 3, 93% depressed, day 6, 67% depressed, day 25 to 26, 16% depressed; only 1 species contained detectable acephate residue; since birds with continuous exposure die at 50% ChE inhibition, this exposure may be life threatening.	NA	Zinkl et al. 1980
Acephate	Junco West bluebird Grace warbler Yellow-rump warbler Pygmy nuthatch Robin	0.85 kg a.i./ha aerial spray (0.75 lb/acre)	1 to 14 days	Brain ChE not inhibited on day 1, but significantly inhibited 2 days after spray; birds with small territories had greatest inhibition; decrease to undetectable residue in brain 14 days; may suffer sublethal effects but unlikely to kill birds.	Acephate metalized to methamidophos.	Zinkl et al. 1984

NA = data not available.

Appendix E

Summary of Acephate Toxicity Data for Mammals

Appendix E

Summary of Acephate Toxicity Data for Mammals

Formulation	Organism	Nature of Exposure	Effects	Comments	Reference
			Acute Oral Toxicity		
Acephate	Rat	Oral. No additional details reported.	LD_{50} - 700 mg/kg	--	Registry of Toxic Effects of Chemical Substances 1981-1982
Acephate (technical)	Rat	Oral. No additional details reported	LD_{50} - 945 mg/kg for males; LD_{50} - 866 mg/kg for females	--	Meister 1984
Acephate	Rat (inferred)	No details reported. Oral route assumed.	No leg paralysis or other signs of delayed neurotoxicity observed at at 375 mg/kg	--	U.S. Environmental Protection Agency 1982
Acephate (technical)	Rat	Oral. No additional details reported.	LD_{50} - 1,800 mg/kg	--	Fukuto 1983
Acephate (purified)	Rat	Oral. No additional details reported.	LD_{50} - 1,480 mg/kg	--	Fukuto 1983
Acephate (technical)	Mouse	Oral. No additional details reported.	LD_{50} - 500 mg/kg	--	Fukuto 1983
Acephate (purified)	Mouse	Oral. No additional details reported.	LD_{50} - 425 mg/kg	--	Fukuto 1983
Acephate (purified plus 5% impurities)	Mouse	Oral. No additional details reported.	LD_{50} - 520 mg/kg	--	Fukuto 1983

Appendix E (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	Reference
Acute Oral Toxicity (Continued)					
Acephate (purified plus specific impurities)	Mouse	Oral. No additional details reported.	LD_{50} = 570 mg/kg for acephate; LD_{50} = 720 mg/kg for acephate plus 1% O,O-dimethyl N-acetylphosphoramidothioate; LD_{50} = 540 and 460 mg/kg for acephate plus 0.5% and 1% O,O,S-trimethylphosphorodithioate, respectively; LD_{50} = 570 mg/kg for acephate plus 5% methamidophos.	--	Fukuto 1983
Acephate (technical)	Mouse	Oral. No additional details reported.	LD_{50} = 500 mg/kg for "unstored" acephate; LD_{50} = 640 mg/kg for acephate stored for 3 mos at 20 °C; LD_{50} = 720 mg/kg for acephate stored for 6 mos at 20 °C; LD_{50} = 780 mg/kg for acephate stored for 3 mons at 40 °C. LD_{50} = 820 mg/kg for acephate stored for 6 mos at 40 °C.	Study designed to determine the effect of storage of acephate for 0, 3, and 6 mos at 20 or 40 °C on its acute toxicity. The conc. of O,O-dimethyl-N-acetylphosphoramidothioate, an impurity and an antagonist of esterase cleavage of acephate to methamidophos, decreased from 0.44% to 0.27% after 6 mos at 40 °C. The toxicity of acephate to house flies was unaltered by the storage condition of the study.	Fukuto 1983

Appendix E (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	Reference
Acute Oral Toxicity (Concluded)					
Acephate	Mouse	Oral. No additional details reported	LD_{50} = 233 mg/kg	--	Registry of Toxic Effects of Chemical Substances 1981-1982
Acephate	Dog	Oral. No additional details reported.	LD_{50} = 581 mg/kg	--	Registry of Toxic Effects of Chemical Substances 1981-1982
Acute Dermal Toxicity					
Acephate	Rabbit	Dermal. No additional details reported.	LD_{50} = 2,000 mg/kg	--	Meister 1984
Acephate	Rabbit	Dermal. No additional details reported.	LD_{50} = >10,250 mg/kg	--	Meister 1984
Subchronic Toxicity					
Acephate	Rat	Given 5 ppm and other unspecified doses in food for 90 days. No additional details available.	NOEL = 0.25 mg/kg (5 ppm in food) based on the inhibition of ChE activity in plasma, red blood cells, and brain tissue.	--	U.S. Environmental Protection Agency 1982

Appendix E (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	Reference
			Subchronic Toxicity (Concluded)		
Acephate (75 SP)	Rat	Given oral doses of 600 mg/kg/day diluted in water for 2, 4, 6, or 8 wks. Control group received no acephate. Animals were given food ad lib. except for 18 hrs before their decapitation at end of treatment.	Slight increases in total liver lipid observed after 4, 6, and 8 wks of treatment. Small, but statistically significant decreases in serum total lipids and triglycerides were observed. No other systematic toxicity effects were reported.	Acephate used had oral LD_{50} of 1,494 mg/kg in rats. Interference with hepatic synthesis and/or secretion of lipoproteins was postulated as the mechanism by which acephate affects the conc. of lipids in both liver and serum.	Choudhari and Chakrabarti 1984
Acephate (75 SP)	Rat (6 per group)	Given oral doses of 60 mg/rat/day for 30, 45, or 60 days (rats weighed 100 to 150 g each). Control group received no acephate. Animals were given food ad lib. throughout study.	In groups treated for 60 days, specific activity of succinic dehydrogenase was significantly decreased ($p<0.05$) and glutathione levels in blood and liver were significantly decreased ($p<0.05$ and $p<0.02$). Total lactic dehydrogenase levels in the 60-day treatment group were significantly elevated ($p<0.05$). No systemic toxicity effects were reported.	Aacephate formulation used had an oral LD_{50} of 1,494 mg/kg in rats.	Patel and Chakrabarti 1982
			Chronic Toxicity		
Acephate	Rat	Given 5 ppm and other unspecified doses in food for 28 mos. No additional details available.	NOEL = 0.25 mg/kg (5 ppm in food) based on inhibition of ChE activity in plasma, red blood cells, brain tissue.	--	U.S. Environmental Protection Agency 1982

Appendix E (Concluded)

Formulation	Organism	Nature of Exposure	Effects	Comments	Reference
			Chronic Toxicity (Concluded)		
Acephate	Dog	Given 30 ppm, 100 ppm, and other unspecified doses in food for 2 yrs. No additional details available.	NOEL = 0.75 mg/kg (30 ppm in food) based on the inhibition of ChE activity in plasma, red blood cells, and brain tissue. NOEL = 2.5 mg/kg (100 ppm in food) based on systemic toxicity. No additional details available.	--	U.S. Environmental Protection Agency 1982
Acephate	Humans	Occupational exposure to acephate in a manufacturing plant and in a lab where large batches were formulated. No additional details available.	Acephate concs. of up to 5 ppm were detected in urine no adverse health health effects and no inhibition of ChE activity in the blood. was detected.	Blood ChE levels are considered a sensitive indicator of organophosphate exposure.	Willcox and Coffey 1977 cited in USDA Forest Service 1984
			Reproductive and Teratogenic Effects		
Acephate	Rat	Highest dose tested was 200 mg/kg. No additional details available.	NOEL ≥ 200 mg/kg for teratogenic effects.	--	U.S. Environmental Protection Agency 1982
Acephate	Rabbit	Highest doest tested was 10 mg/kg. No additional details available.	NOEL ≥ 10 mg/kg for teratogenic effects.	--	U.S. Environmental Protection Agency 1982

Appendix F

Summary of Neurotoxicity and Behavior Data for Acephate

(No data currently available)

Appendix G

Summary of Mutagenicity Data for Acephate

Appendix G

Summary of Mutagenicity Data for Acephate

Formulation	Organism	Nature of Exposure	Effects	Comments	Reference
Acephate	Salmonella typhimurium (TA100)	Details unspecified. Reverse point mutation assay without metabolic activation.	Reported to induce 0.0013 revertants per nana-mole (details unspecified).	--	Shirasu et al. 1981
Acephate	S. typhimurium (TA1535, TA1537 TA1538, TA100)	Dosed at unspecified levels with and without S9 metabolic activation. Reverse point mutation assay.	Weakly mutagenic in TA100. No detectable mutagenic activity in the other strain tested.	TA100 detects base-pair substitution events.	Waters et al. 1980
Acephate	S. typhimurium (TA1535, TA1537, TA1538, TA98, TA100)	Dosed at five concentration up to 40,000 µg/plate with and without S9 metabolic activation. Reverse point mutation assay.	Mutagenic with and without S9 metabolic activation in TA100 but not in any other strain tested.	TA100 detects base-pair substitution events.	Moriya et al. 1983
Acephate (Orthene 75 SP)	S. typhimurium (hisC117, hisG46, TA1530, TA1535)	Details not reported. Reverse point mutation assay without S9 metabolic activation.	Not mutagenic; no significant increase over the spontaneous reversion frequency. Details not reported.	--	Hanna and Dyer 1975
Acephate (nitrosated in vitro with sodium nitrate)	S. typhimurium (hisG46)	Dosed at 1, 10, and 100 µl/plate without S9 metabolic activation. Reverse point mutation assay (spot test).	Not mutagenic; no significant increase spontaneous revertion frequency.	--	Seiler 1977
Acephate	S. typhimurium (SL4525 and SL4700)	Details not available. Differential toxicity assay.	Not mutagenic; no significant differences in toxicity between strains. Details not reported.	Strain SL4525 is DNA repair-proficient while strain SL4700 is DNA repair-deficient.	Waters et al. 1982

Appendix G (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	Reference
Acephate (Orthene 75 SP)	Escherichia coli (WP2, WP2uvrA, CM561, CM571, CM611, WP67, WP12)	Details not reported. Reverse point mutation assay (spot test) without activation.	Mutagenic in strains WP2, WP2uvrA, WP67, and WP12. Details not specified.	All strains used detect base-pair substitution events	Hanna and Dyer 1975
Acephate	E. coli (WP2 hcr)	Details not available.	Mutagenic (details not available).	WP2 hcr detects base-pair substitution events.	Moriya et al. 1983
Acephate	E. coli (WP2)	Dosed at unspecified levels with and without S9 metabolic activation. Reverse point mutation assay.	Not mutagenic; no increase over spontaneous reversion frequency.	--	Waters et al. 1980
Acephate	E. coli (W3110 and P3478)	Dosed at unspecified levels. Differential toxicity assay.	Inconclusive; no growth inhibition observed in either strain.	Strain W3110 is repair-proficient; strain P3478 is repair-deficient. Authors state that the negative result may reflect inability of acephate to diffuse into agar.	Waters et al. 1980 and 1982
Acephate	Bacillus subtilis (H17 and M45)	Dosed at unspecified levels. Differential toxicity assay.	Inconclusive; no growth inhibition observed in either strain.	Strain H17 is repair-proficient; strain M45 is repair-deficient. Authors state that the negative result may reflect inability of acephate to diffuse into agar.	Waters et al. 1980 and 1982
Acephate	Saccharomyces cerevisiae (D3 and D7)	Details unspecified (abstract).	Reported to induce mitotic recombination in the D3 assay and mitotic crossing-over mitotic gene conversion, and reverse mutation in the D7 assay (details unspecified).	D3 assay allows detection of induction of mitotic recombination. D7 assay allows detection of induction of mitotic crossing-over, mitotic gene conversion, and reverse mutation.	Riccio et al. 1981

Appendix G (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	Reference
Acephate	*S. cerevisiae* (D3)	Dosed at unspecified levels with and without S9 metabolic activation. Mitotic recombination assay.	Induced mitotic recombination in the strain of yeast tested.	--	Water et al. 1980
Acephate	*S. cerevisiae* (D7)	Details not available. Reverse point mutation assay.	Mutagenic with S9 metabolic activation, but not without. Details not reported.	--	Waters et al. 1982
Acephate	*S. cerevisiae* (D7)	Details not available. Gene conversion and crossing-over assay.	Mutagenic; induced gene conversion and crossing-over. Details not reported.	--	Waters et al. 1982
Acephate	Human fetal lung fibroblast (WI-38)	Dosed at unspecified levels with and without S9 metabolic activation. Unscheduled DNA synthesis (UDS) assays.	Mutagenic without S9 metabolic activation; increased UDS over the untreated control values. Not mutagenic with S9 metabolic activation.	--	Waters et al. 1980
Acephate	Mouse lymphoma L5178Y cells	Details not available. Forward point mutation assay.	Mutagenic; details not reported.	--	Waters et al. 1982
Acephate	Chinese hamster ovary cells	Details not available. Sister chromatid exchange (SCE) assay.	Mutagenic; induced sister chromatid exchanges significantly above the baseline SCE frequency. Details not reported.	--	Waters et al. 1982
Acephate	Mouse bone marrow and cardiac blood	Details not available. Chromosome breakage (micronuclei) assay.	Not mutagenic; an insignificant number of blood cells containing micronuclei were observed. Details not reported.	--	Waters et al. 1982

Appendix G (Concluded)

Formulation	Organism	Nature of Exposure	Effects	Comments	Reference
Acephate	Drosophila melanogaster	Adult males were exposed to 10 ppm acephate in their food for 48 hrs before mating at intervals of 4, 3, 3, and 4 days to produce 4 broods of progency. Sex linked recessive lethal assay.	Not mutagenic; response in treated group not significantly different from control values.	Counting day 1 as the first day of mating, the germ cells sampled included 4, 7, 10, and 14-day germ cells.	Valencia 1977
Acephate	D. melanogaster	Details not available; but apparently tested at a dose least 10 ppm for at least 24 hrs. Sex-linked recessive lethal assay.	Not mutagenic; details not available.	--	Waters et al. 1980

Appendix H

Summary of Studies With Acephate and Metabolism

Appendix H

Summary of Studies With Acephate and Metabolism

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Acephate (Monitor and metabolites)	Cotton	Singular foliar application	48 hrs	>50% absorbed by leaves. Acetate metabolized to 9% of methamidophos and four other products.	Unabsorbed residue depleated in 48 hrs.	Bull 1979
Acephate (98.5%)	NA	Mitochondria lab exposure	--	Acephate may combine with cytochrome C oxidase and modify the configuration of the enzyme.	--	Ando and Wakamatsu 1983

NA = data not available.

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Bacillus thuringiensis Summary

Major Trade Names: Dipel®, Vectobac®, Thuricide®, Certan®, Bactimos®, Futura®, Javelin®, Teknar®, Leptox®, Bug Time®, Attack®, and others.

Chemical Names: No chemical name. Formulations are derived from various strains of the *Bacillus thuringiensis* bacterium. The USDA Forest Service uses only formulations derived from *B. thuringiensis* var. *kurstaki*.

Major Forestry Applications: Control of eastern and western spruce budworm, gypsy moth, bagworm, tent caterpillars, western and Douglas-fir tussock moth, hemlock looper, jack pine budworm, and other hardwood defoliators.

Detailed information on toxicity, environmental fate, and hazard assessment can be found in the body of the pesticide background statement.

The toxicological effects of *B.t.* formulations are due to several biologically active metabolic products of the bacterium *Bacillus thuringiensis* (*B.t.*). The alpha-exotoxin is an unidentified protein that is toxic to insects and vertebrates at high concentrations. It is produced during the vegetative growth phase of *B.t.* but is itself not a component of commercial formulations derived from *B.t.* Formulations derived from some varieties of *B.t.* (but not from var. *kurstaki*) contain a heat-stable nucleotide, the beta-exotoxin, that is toxic to both vertebrates and invertebrates. The delta-endotoxin is a proteinaceous material produced during spore formation by the bacterial cell. The delta-endotoxin is particularly toxic to larvae of lepidopteran insects (butterflies and moths), coleopterans (beetles), and to some dipterans (flies and mosquitos). The delta-endotoxin is the basis for most commercial formulations. In addition to these three, there are several other toxins associated with *B.t.*, including various enzymes collectively known as gamma-exotoxins as well as; proteinaceous extracellular compounds that inhibit the immune system of pupae of some moths and the fruit fly and an entity that is toxic to mammal-biting lice.

Toxicology

Field studies indicate there are little to no adverse impacts to nontarget species, including worms, honey bees, and aquatic benthos, following use of *B.t.* Bird and mammal populations were also unaffected by its application. The insecticidal activity of the delta-endotoxin in the gut of insects occurs when the parasporal crystalline material is degraded under alkaline conditions. There are no available studies dealing with the toxic effects on microorganisms of *B*.*t*. formulations from which the beta-exotoxin is absent. Available studies indicate that field applications of *B*.*t*. at normal and excessive rates of application do not appear to have any adverse effects on nontarget invertebrate species as long as the formulations used do not contain toxic entities other than the delta-endotoxin. Field studies using Thuricide and Dipel and laboratory tests at Abbott indicate that *B*.*t*. is not toxic to fish. Available studies indicate that the delta-endotoxin is practically nontoxic to birds.

B.*t*. formulations currently used by the USDA Forest Service are generally nontoxic to mammals, including humans, since the toxic alpha-exotoxin is denatured during the manufacturing process and the strains that are used do not synthesize beta-exotoxin. Studies indicate that *B*.*t*. is not acutely toxic to mammals as long as the pesticidal formulations do not contain these two toxins. Subchronic toxicity tests indicate no toxic effects from oral exposure and no evidence of dermal toxicity or sensitization. No chronic toxicity was observed in rats fed 1 percent *B*.*t*. in the diet for 2 years or in workers exposed to *B*.*t*. for as long as 10 years. There are no data available in the literature indicating reproductive or teratogenic effects of *B*.*t*. formulations or indicating its possible carcinogenicity. There are no reports of neurotoxic effects from *B*.*t*. formulations. Studies on the mutagenicity of *B*.*t*. indicate that it is nonmutagenic.

Environmental Fate

There is limited information on the environmental fate and behavior of vegetative cells of *B*.*t*. or of its spores and crystals. *B*.*t*. is a naturally occurring pathogenic organism normally found in the environment at levels lower than those commonly found compared to levels that occur after application of pesticidal formulations. The spores and crystals appear to be subject to environmental degradation as a result of solar radiation, temperature, and vapor pressure. Vegetative cells are subject to bactericidal action by several sources. The persistence of vegetative cells and of activity of the various toxins of *B*.*t*. is strongly dependent on the specific nature of the formulation used and on various environmental factors of sunlight, humidity, and edaphic factors. The persistence of *B*.*t*. on plants varies, with specific factors of plant morphology affecting the patterns of initial spore deposition and with bactericidal activity of fluids of some plants affecting viability of the bacterium. *B*.*t*. does not persist in the gut of vertebrates, but both spores and cells can remain viable for as long as 8 weeks in fecal material. B.t. formulations are moderately persistent in most soil environments. There are no reports in the literature regarding the behavior and fate of B.t. formulations and of specific toxins such as the delta-endotoxin in aquatic systems. One report for behavior of B.t. in the atmosphere indicated persistence in the air for as long as 17 days after aerial application of B.t. var. thuringiensis to a mixed conifer forest.

Exposure and Hazard

Available data indicate that the B.t. formulations are used by the USDA Forest Service, derived from B.t. var. kurstaki, have a low toxicity to vertebrates and to other nontarget organisms and are not mutagenic. There should be no significant potential for adverse toxic, mutagenic, reproductive, or carcinogenic effects to individuals from use of formulations derived from B.t. var. kurstaki at levels of application used by the USDA Forest Service. Care should be taken during use, since several studies have reported isolation of vegetative cells and spores of B.t. from a skin infection and from an eye ulcer following accidental occupational exposure to B.t. formulations. Therefore, warning labels caution to avoid inhalation, contact with eyes, and open wounds.

1.0 General Information

This pesticide background statement differs slightly from the others in these series, since *Bacillus thuringiensis* (*B.t.*) formulations are based on a biological agent rather than a chemical agent. This difference necessitates additional information about the biology of the bacterium *Bacillus thuringiensis* and about the nature and measurements of its toxic agents. Much of this information is discussed in the beginning of this general information section.

This document discusses primarily the toxicology and environmental fate of *B.t.* var. *kurstaki* (HD-1), since this strain is used to produce the commercial *B.t.* formulations used for USDA Forest Service applications. *B.t.* var *kurstaki* meets the needs of the USDA Forest Service for a narrow-spectrum insecticide that is primarily effective against lepidopterans.

The literature on which this pesticide background statement is based varied with regard to the units of measurement used to quantify toxicity. Generally these units were expressed in terms of toxic potency, relative activity. or application rates. Specifically, the units employed included IU (International Units)/mg, BIU (Billion International Units) per pound (also BIU per quart or BIU per liter), number of viable spores/unit weight or unit volume, unit weight or unit volume applied/unit area, and percent active ingredient formulation. In many cases, neither the variety or strain of *B.t.* used, nor the nature or strength of the specific toxic entity, were indicated. Such omissions make interpretation of the data difficult. Where possible in this background statement, appropriate conversions have been made. Assumptions that have been made in the background statement about the nature of the formulation employed are indicated. Often these assumptions were based upon context of the referenced article.

B.t. is a rod-shaped, aerobic, spore-forming bacterium approximately 1.0 to 1.2 μm in diameter and 3 to 5 μm long (Dulmage and Beagle 1982, Huber and Luthy 1981). It is a naturally occurring soil pathogen. The bacterial family Bacillaceae comprises only two genera, *Bacillus* and *Clostridium*. This family is characterized by environmentally resistant endospores (small asexual spores) that are formed at the end of a period of vegetative growth. As a species, *B.t.* is further characterized by a distinctive parasporal body, a crystal that is found in mature, sporulated, normal cells. Because of the presence of this crystal, there is frequent reference in the scientific literature to the various varieties of *B t.* as the crystalliferous bacteria.

The bacterium was first isolated in 1902 from silkworm larvae in Japan and initially described and named as Bacillus sotto. Berliner (1915, in Luthy and Ebersold 1981) isolated and described this same bacterium from diseased Mediterranean flour moths and named it Bacillus thuringiensis. Fifty years later, the bacterium was proposed for use as an insecticidal agent against the alfalfa caterpillar (Hayes 1982). It has since been used alone and in combination with nuclear polyhedrosis virus, fungi, fungicides, insecticides, herbicides, or acaricides in a variety of integrated pest control systems. Its early use in the United States was for control of the cabbage looper, Trichoplusia ni (Bulla et al. 1975, Luthy and Ebersold 1981). At the present time, insecticidal formulations derived from B.t. are used more widely than those derived from any other microorganism for control of insect pest species in forests (Luthy and Ebersold 1981).

The efficacy of B.t. as an insect pathogen has been recognized since the early 20th century. Numerous reviews have summarized the biology of B.t. and the efficacy and toxicity of its various formulations (Angus 1971, Bulla et al. 1975, Dulmage et al. 1981, Forsberg et al. 1976, Huber and Luthy 1981, Luthy and Ebersold 1981, Sebesta et al. 1981).

Originally, it was the bacterium itself that was considered the insecticidal agent. Subsequent research indicated that the insecticidal properties of B.t. are due to metabolites (toxins and protoxins) produced by the bacterium, one group of which are associated specifically with the parasporal body or crystal (Dulmage and Beegle 1982). Different metabolites, produced during various stages of the life cycle of B.t. have been shown to be toxic to various insect species, primarily to larvae of the order lepidoptera (moths and butterflies). The different strains of B.t. produce a variety of toxic compounds. Production of these toxic compounds is primarily strain-specific, but is also determined to some extent by environmental factors. The specific toxic substances produced by various strains of B.t. are described in section 1.2.3.

The toxic compounds produced by the different strains of B.t. have varying effects on specific insect pests. Consequently, a variety of pesticidal formulations have been developed for agricultural and forest insect control applications, based on one or more strains of the bacterium. Several available formulations of B.t. are described in table 1-1.

In the United States, available formulations are derived from three strains of B.t. (Meister Publishing Company 1984). The EPA currently has registered 400 products containing B.t. to 94 companies (U.S. Environmental Protection Agency 1985). Formulations derived from

Table 1-1

Bacillus thuringiensis Formulations

Formulation	Active Ingredient Billion International Units[a]	Formulator
B.t. var. *kurstaki*		
Dipel (WP)	7.26/lb	Abbott Laboratories, Chemical and Agricultural Products Division
Dipel 4L	32/gal	Abbott Laboratories, Chemical and Agricultural Products Division
Dipel 2X	14.52/lb	Abbott Laboratories, Chemical and Agricultural Products Division
Dipel 6L	48/gal	Abbott Laboratories, Chemical and Agricultural Products Division
Dipel 8L	64/gal	Abbott Laboratories, Chemical and Agricultural Products Division
Dipel 8AF	64/gal	Abbott Laboratories, Chemical and Agricultural Products Division
Dipel 6AF	48/gal	Abbott Laboratories, Chemical and Agricultural Products Division
Thuricide 32LV	8/qt	Sandoz Crop Protection Corp.
Thuricide 48LV	12/qt	Sandoz Crop Protection Corp.
Thuricide 64LV	16/qt	Sandoz Crop Protection Corp.

Table 1-1 (Continued)

Formulation	Active Ingredient Billion International Units[a]	Formulator
B.t. var. kurstaki (cont'd)		
Javelin	8/qt	Sandoz Crop Protection Corp.
SAN 415 SC32LV	8/qt	Sandoz Crop Protection Corp.
Certan	NA	Sandoz Crop Protection Corp.
SOK-BT	NA	TUCO, Division of Upjohn Co.
Sod Webworm Attack	16,000	Reuter Laboratories, Inc.
Caterpillar Attack	16,000	Reuter Laboratories, Inc.
B.t. var. aizawai		
Certan	NA	Sandoz Crop Protection Corp.
B.t. var. israelensis		
Vectobac	2.275/lb	Abbott Laboratories, Chemical and Agricultural Productss Division
Vectobac TP (technical powder)	2.27/lb	Abbott Laboratories, Chemical and Agricultural Products Division
Vectobac G	0.091/lb	Abbott Laboratories, Chemical and Agricultural Products Division
Vectobac 12AS	2.19/gal	Abbott Laboratories, Chemical and Agricultural Products Division

Table 1-1 (Concluded)

Formulation	Active Ingredient Billion International Units[a]	Formulator
B.t. var. *israelensis* (cont'd)		
Vectobac AS	2.19/gal	Abbott Laboratories, Chemical and Agricultural Products Division
Vectobac WP	2,000 AA[b]/mg	Abbott Laboratories, Chemical and Agricultural Products Division
Bactimos		
-- Granule	175 AA/mg	PBI/Gordon
-- Wettable Powder	3,500 AA/mg	PBI/Gordon
-- Flowable Concentrate	1,000 AA/mg	PBI/Gordon
-- Pellets	350 AA/mg	PBI/Gordon
-- Rings	350 AA/mg	PBI/Gordon
Bactimos Briquets	700 AA/mg	Summit Chemical
Mosquito Attack Spray	3,500 AA/mg	Reuter Laboratories, Inc.
Skeetal Flowable	1,400 AA/mg	Unknown
Teknar 2X	8/qt	Sandoz Crop Protection Corp.

[a] International comparison units for activity. Example: 16.8 BIU equal potency of 16,000 IU (at least 24 million viable spores)/mg.

[b] *Aedes aegypti* for mosquitoes only.

NA = data not available.

Source: Forsberg 1976, Gosselin 1976, Meister Publishing Company 1984, Abbott 1986, Sandoz 1986, Environmental Protection Agency 1985.

B.t. var. aizawai (serotype H-7) are based on spores and crystals and are known by the trade name Certan. They are primarily used for control of wax moth larvae in honeycombs. Formulations derived from B.t. var. israelensis (serotype H-14) contain the parasporal crystal and its associated delta-endotoxin and are known by trade names such as Bactimos, Teknar, and Vectobac, as well as by BMC. These formulations are particularly effective in controlling mosquito and blackfly larvae. Formulations derived from B.t. var. kurstaki (serotype H-3a, 3b; HD-1), containing both spores and the parasporal crystal with its associated delta-endotoxins as the toxic compounds, are known by trade names such as Bactur®, Dipel, SOK®-Bt, and Thuricide, as well as by Tribactur® and several discontinued trade names. The formulations of B.t. var. kurstaki (serotype 3a, 3b) used by the Forest Service are produced from the HD-1 strain and contain no exotoxin (Abbott Laboratories 1985). These formulations are particularly effective against lepidopteran insect larvae with high gut pH, such as spruce budworm, gypsy moth, and cabbage looper.

Commercial formulations based on B.t. contain toxic substances associated with both spores and crystals. In the United States and Canada, commercial formulations of B.t. must be free of beta-exotoxin, an ATP analogue toxic to both invertebrates and vertebrates (Brown 1975, in Forsberg et al. 1976). The formulator can either remove the beta-exotoxin while harvesting spores and crystals or can use a strain, such as B.t. var. kurstaki, which does not produce the beta-exotoxin. Formulations that are currently used for USDA Forest Service applications, such as Dipel and Thuricide, are prepared from the kurstaki variety and thus are free from the beta-exotoxin (Forsberg et al. 1976).

B.t. is found under natural conditions in the environment, although it is not encountered frequently (Angus 1968, Luthy and Ebersold 1981). Normally, infestation by B.t. does not result in high levels of mortality among infected insect populations. Naturally occurring epizootic outbreaks are rare. The level of mortality due to naturally occurring B.t. is lower than mortality resulting from application of pesticidal formulations. This lower mortality is due to the relatively large numbers of vegetative cells, compared to spores and crystals containing the delta-endotoxin, among naturally occurring populations of B.t. Commercial production of B.t. fosters the production of spores and crystals by aerobic culture techniques. In infected insects, the relatively low oxygen content of the tissues promotes production of vegetative cells at a greater rate than production of spores and crystals, thus preventing the rapid build-up of the specific agents of mortality in an infected insect population.

The available data on environmental fate of B.t. indicate that its persistence is quite variable in most environmental media, depending to a large extent on specific environmental factors (Forsberg et al. 1976). It does not persist in aquatic systems or in the gut of vertebrates following cessation of ingestion. In soils, persistence of spores and cells of B.t. is increased (not quantified in literature) by increased nutrients and by higher pH. Spores are capable of remaining viable in soils for several months under suitable conditions. When exposed to atmosphere, there was a 90 percent reduction in B.t. spores within 4 months (Forsberg et al. 1976).

1.1 Normal Use Patterns

Any discussion of normal use patterns for B.t. requires an understanding of how formulations are standardized. The history and rationale behind the development of standardization of the different formulations derived from B.t. are discussed in Dulmage et al. (1971), Dulmage (1973a and 1973b), Burgerjon and Dulmage (1977).

Actual comparison of the relative activities of two or more different formulations is difficult for several reasons: different formulations may be derived from different strains that have produced chemically and biologically different toxic compounds; these different toxic compounds may have different biological effects on any given organism; and the different formulations may have different concentrations of either spores or toxic compounds, or both.

Current schemes for standardization of B.t. formulations are based on a standard reference preparation that was produced from a serotype 1 B.t. strain. This standard reference, a formulation produced by Abbott in 1980, is identified as HD-1-S-1980. It was arbitrarily assigned an activity titer of 1,000 "biological units"/mg, with a potency of 16,000 IU/mg. Most commercial formulations produced in the United States, however, are derived from the HD-1 (serotype 3a, 3b) isolate of B.t. var. kurstaki, which produces a relatively high yield of delta-endotoxin compared to other isolates. In the United States, these commercial formulations are compared to a reference preparation, designated as HD-1-S-1971, also produced from the HD-1 isolate (in 1971). This comparison of toxic activity of the various commercial formulations is based on a bioassay that uses the cabbage looper, Trichoplusia ni, as the test insect. In a series of tests reported by Dulmage (1973b) and Burgerjon and Dulmage (1977), the previous U.S. standard reference, HD-1-S-1971, was found to be 18 times more active against the cabbage looper in terms of toxicity than was the 1961 French standard, E-61. Therefore, the HD-1-S-1971 standard reference preparation was assigned a potency value of 18,000 IU/mg. Activity is expressed as International Units (IU), and application rates are commonly given as BIU (Billion International Units) per acre, or per some other unit area. The term "international" indicates international comparison. Although the 18 to 1 relationship does not hold for all other species of insects, the cabbage looper tests provide a standard for comparison of the toxicity of the various formulations derived from B.t. However, it must be remembered that potency varies between insect species.

Application rates for B.t. formulations are expressed in terms of specific activity, which in turn is a function of the toxicity of the specific formulation to target organisms. This activity is usually determined by bioassay techniques (such as that employing the cabbage looper) in which the toxicity of a commercial formulation is compared to that of a reference formulation in a specified test organism.

Within the text, tables, and appendices of this pesticide background statement, reasonable attempts have been made to provide as much information as possible about the specific activity of the formulations referenced. However, many studies (1) do not indicate the activity of the formulation in International Units; (2) do not specify the strain, isolate, or variety of B.t. from which the formulation was derived; or (3) provide information regarding activity only in terms of viable spores/volume or weight of formulation. In these instances, it is difficult to compare results with those from studies in which the activity of the formulation has been provided in terms of International Units.

Formulations of B.t. are used by the USDA Forest Service for control of forest insect pests such as the spruce budworm and gypsy moth. In 1983, insecticidal formulations derived from B.t. were applied on 12,492 acres for control of the western spruce budworm and on 75 acres for control of unspecified hardwood defoliators (Gross 1985). From 1984 to 1986, more than 2 million acres were sprayed with Dipel (Abbott 1986). These formulations were applied by aerial spray at a rate of 8-20 BIU/acre. Data on use of B.t. formulations by the USDA Forest Service in 1983-1986 are presented in table 1-2. Non-USDA applications account for 90 percent of B.t. forestry use in the U.S. (Sandoz 1986).

In the field, B.t. is used for gypsy moths and is usually sprayed at rates ranging from 12 to 20 BIU/acre, with 16 BIU considered a normal application rate (96 oz/acre). Success rates vary depending upon when the spray was applied, whether repeat applications were used, and the type of weather conditions. For example, the success of the 1984 B.t. spray program in Oregon resulted in use of only B.t. in 1985. On the other hand, the New Jersey program resulted in only a 21 percent larval reduction in 1985, a number of complaints about B.t., and a need

Table 1-2

USDA Forest Service *Bacillus thuringiensis* Use 1983-1986

Purpose	Active Ingredient/Acre (BIU) Range	Active Ingredient/Acre (BIU) Average	Acres Treated	Formulation	Application Method
1983					
Western Spruce Budworm Control	8-20	14	12,492	Liquid	Aerial Spray
Hardwood Defoliator Control	8-20	16	75	Liquid	Aerial Spray
1984					
Gypsy Moth Control	8-20	16	24,000	Liquid	Aerial Spray
1985					
Western Spruce Budworm Control	8-20	14	99,099	Liquid	Aerial Spray
Gypsy Moth Control	8-20	16	73,590	Liquid	Aerial Spray
1986					
Gypsy Moth Control	8-20	16	1 million (estimate)[a]		Aerial Spray

[a] Schneeberger (1986) estimate 1980-1986 USFS use on 1,827,696 acres FS Cooperative programs.

Source: Cross 1985, USDA Forest Service 1985, Wolfe 1985.

to retreat 75 percent of the areas in 1986 (Thom 1985). Pennsylvania B.t. treatments were applied at 12 BIU/acre over 70,882 acres, and up to 20 BIU/acre over 29,722 acres. The average gypsy moth reduction was 89 percent in treated areas; while untreated areas reported a 148 percent increase in gypsy moths (Slippey 1985).

In Oregon, over a quarter of a million acres, were sprayed with Dipel 8L (16 to 20 BIU/acre) and gypsy moths were eradicated (following four applications) in 13 of 18 spray blocks. Youngs (1985) concludes with the following findings for B.t. use on gypsy moths:

Advantages

- Very specific to target, only lepidopteran larvae affected
- No adverse human health effects, with the exception of two undetailed incidences
- Applications over rivers and lakes permitted
- At high densities, considerable reduction; at low densities population eliminated

Disadvantages

- Residual moth population following spray
- Increased costs for multiple application
- No contact activity, dispersing moths unaffected until they feed
- Disrupt avian food chain
- Public concerns over human health
- Poor performance in adverse weather

The actual amount of active ingredient that is applied during field applications of B.t. formulations is often difficult to quantify, yet this information is important in understanding both the efficacy of these formulations and their toxic effects on nontarget organisms. Several studies in the literature address the efficacy of B.t. formulations against forest insect pest species. In some instances, it is difficult to adequately interpret the results of these studies due to lack of detailed information concerning the specific activity applied per unit area. As with all pesticides, effective aerial application of B.t. formulations requires accurate and precise delivery of the biologically active material to the target. Several methods used to calibrate aerial delivery systems were described by Smirnoff (1979, 1980a, 1980b) and Smirnoff and Juneau (1979) in studies of the efficacy of B.t. formulations for control of spruce budworm in Quebec. In one technique, petri dishes with nutrient agar were placed in the flight path of the spray craft and bacterial colonies were then counted after 15 to 24 hours of incubation. In the other, 10 by 10 cm Kromekote cards were placed in the flight path of the spray aircraft and the number of droplets/square centimeter (cm^2), as well as volume of individual droplets, were determined by electronically scanning the deposits on the cards. Abbott (1986) recommends the following:

> In operating or experimental spray programs, it is often desirable to monitor spray deposit either on artificial surfaces (spray cards) or on the actual target foliage. In order to effectively dye DIPEL formulations and not alter the physical characteristics (viscosity) of the formulations, it is necessary to utilize the following recommended spray dye materials: (1) for undiluted DIPEL, Keystone Liquid Oil Red; (2) for diluted DIPEL, Rhodamine B powder or Rhodamine B (40% solution).
>
> Spray cards or foliage should be read immediately following applications, since DIPEL formulations are oil-based and the spray deposits will tend to migrate on the target surface with time.
>
> Several types of deposit collectors have been successfully utilized to monitor spray deposition with DIPEL formulations. These include white Kromekote cards, water-sensitive cards, and oil-sensitive cards.
>
> For diluted applications, without the addition of spray dye, either water or oil-sensitive spray cards can be utilized. For undiluted applications, without the addition of spray dye, only oil-sensitive spray cards should be utilized. For dyed undiluted or diluted tests Kromekote cards can be utilized.

1.2 Physical and Chemical Properties

B.t. is a biological agent and, as such, has a biological relationship with the environment in addition to the chemical actions of its metabolites. The following discussion of the physical and chemical properties of B.t. addresses the biology of the bacterium both in terms of its life cycle and the biological and chemical nature of the major toxic products of the various strains of B.t. Section 1.2.3 briefly reviews studies in which effects on B.t. itself have been reported as a result of pesticides used along with B.t. formulations. This information is included to illustrate the potential for loss of, or increase in. activity of B.t. depending upon the use of other pesticidal formulations.

Numerous varieties or strains of B.t. are described and reviewed in the literature. A total of 28 taxonomic varieties are classified, according to serological and biochemical differences of their flagella, into 14 different serotypes (DuBois 1986). Of the 14 generally accepted serotypes, serotypes 1 through 13 have toxic effects that are primarily confined to lepidopteran insects. Serotype 14 is highly toxic to the larvae of mosquitos and blackflies (insect order Diptera) (Luthy 1980). The diversity of the different serotypes is illustrated by the fact that, as of 1982, nearly 800 genetic isolates of B.t. were available from the USDA Agricultural Research Service (Dulmage and Beegle 1982).

1.2.1 Life Cycle

Several articles, including those by Angus (1971), Forsberg et al. (1976), Luthy and Ebersold (1981), have described the life cycle of B.t. Essentially, there are two phases to the life cycle of the bacterium. The first is a vegetative growth phase that is characterized by rapid cell proliferation. During the second phase, spores that have been formed at the end of the vegetative stage lie dormant until they infect a suitable host. As described by Angus (1971), spores of B.t., when placed into a suitable medium such as a laboratory culture or appropriate host insect tissues, germinate and produce rod-shaped vegetative cells. These vegetative cells proliferate, excreting exoenzymes that digest the surrounding media or tissues and are toxic to the host insect. The vegetative phase is characterized by vigorous cell proliferation, after which, possibly due to depletion of nutrients and increasing levels of metabolic by-products, the vegetative cells form and release their spores. In addition, various toxic byproducts of the metabolism of the vegetative-phase bacteria may also be produced. At the same time as the spores are formed, the cells produce, over a relatively short time period, a proteinaceous inclusion body. This structure is usually referred to either as the parasporal body or as the crystal. According to Prasad and Shethna (1976), the mass of this crystal amounts to as much as 30 percent of the dry weight of the mature bacterium.

1.2.2 Toxins Produced by *Bacillus thuringiensis*

Dulmage et al. (1981), Forsberg et al. (1976), Luthy (1980), Luthy and Ebersold (1981), among others, reviewed in detail the nature of the various toxins produced by B.t. strains. These biologically active metabolic products have been categorized on the basis of their chemical nature and on the nature and timing of their synthesis during the life cycle of the bacterium.

1.2.2.1 Alpha-exotoxin. The alpha-exotoxin, also known as mouse-factor (Krieg 1971, in Forsberg et al. 1976), is an unidentified protein that at relatively high concentrations (no range indicated) is toxic to insects and to vertebrates. However, alpha-exotoxin does not appear in commercial formulations derived from B.t. and therefore is not of concern in most studies. There is, however, the potential for production of alpha-exotoxin by vegetative bacterial cells in infected insects (Luthy 1980).

There is little information concerning the alpha-exotoxin (Luthy 1980). It has been reported to be a heat-labile protein produced by vegetative stage bacterial cells of B.t. during the logarithmic growth phase, but only under certain culture conditions (Krieg and Lysenko 1979, in Luthy and Ebersold 1981). It is also produced by strains of Bacillus cereus (Luthy 1980). Neither the specific nature of the protein nor its mode of action have been clearly determined. Toxicity of the alpha-exotoxin is not species specific. It has been reported to be toxic by the oral route in honey bees and in mice (Krieg 1973, in Forsberg et al. 1976).

The alpha-exotoxin does not occur in commercial spore crystal formulations derived from B.t. since it is inactive during the manufacturing process. According to Forsberg et al. (1976), it is uncertain whether or not B.t. var. kurstaki even produces alpha-exotoxin during the vegetative growth phase. If cells of B.t. var. kurstaki do indeed produce the alpha-exotoxin, there could be cause for concern for potential environmental toxicity, since the toxin could be produced by vegetative bacterial cells and accumulate in infected insect larvae.

1.2.2.2 Beta-exotoxin. Formulations derived from prior varieties of B.t. contained the beta-exotoxin, a heat-stable nucleotide that was toxic to both vertebrates and invertebrates, particularly to mosquitos and flies (Diptera). Beta-exotoxin has also been referred to in the literature as exotoxin, fly factor, and as the thermostable exotoxin (Heimpel and Angus 1963, in Forsberg et al. 1976). Serotypes 5a, 5b (var. galleria) and 7 (var. aizawai) also can produce the beta-exotoxin (DuBois 1986). The nature of the beta-exotoxin produced by some strains has been reported by several authors (Lecadet and de Barjac 1981, Luthy 1980, Sebesta et al. 1981, Luthy and Ebersold 1981). In today's commercial preparations, however, the manufacturer must verify that the product *B.t.* does not contain the toxic beta-exotoxin. Therefore, further analysis is not warranted.

1.2.2.3 *Delta-endotoxin*. A third substance produced by *B.t.* is delta-endotoxin, a proteinaceous material associated with the parasporal crystal. The parasporal body is also known as the crystal, proteinaceous crystal, and crystalline parasporal body (Heimpel and Angus 1963, in Forsberg et al. 1976). Delta-endotoxin is particularly toxic to lepidopteran insects (butterflies and moths) and to some dipterans (flies and mosquitos). Delta-endotoxin is the basis for most commercial *B.t.* formulations.

The chemistry of the delta-endotoxin has been studied extensively, since it is the primary insecticidal component of commercial formulations derived from *B.t.* used in the United States. The biological and chemical nature of delta-endotoxin have been reviewed by several authors (Bulla et al. 1981, Fast 1981, Faust et al. 1974, Forsberg et al. 1976, Huber and Luthy 1981, Luthy 1980, Luthy and Ebersold 1981, Pendleton 1976, Prasad and Shethna 1976, Tyrell et al. 1981).

The proteinaceous material of which the crystal or parasporal body is composed is considered to be a protoxin (Luthy 1980). As such, the proteins are not toxic until they have been activated within the gut of the insect. This activation requires release of the protoxin from the crystal. This release occurs only under conditions of high pH. It is followed by proteolytic activity on the part of naturally occurring proteases within the gut fluids. This proteolytic activity serves to break the large proteins into smaller subunits, some of which are toxic.

The crystal is usually bipyramidal in shape and is composed of protein subunits that are dimers having a molecular weight of 230,000 daltons. These subunits are rod shaped and are approximately 5 by 15 to 9 by 13.5 nanometers (nm) in size (Norris 1971, Holmes and Monroe 1965, both in Fast 1981, Luthy and Ebersold 1981). Estimates of both the molecular weight of the dimer protein subunit and the weight of the monomer that is derived following denaturation of the dimer differ, depending upon the method of weight determination. Values for the molecular weight are consistently lower when determined by ultracentrifuge than by other means (Huber and Luthy 1981, Luthy and Ebersold 1981).

The potential toxicity of the parasporal crystal is maintained despite exposure to a variety of environmental extremes. Burges et al. (1975) reported that the toxic effect of crystals, measured in terms of inhibition of feeding by large cabbage white butterfly larvae, was not altered following gamma-irradiation of crystals with 3.0 Mrad at 37.4 °F (3 °C). Nor was the effect of the crystals on feeding inhibition affected by ultraviolet radiation under a germicidal UV tube for 9 hours at 68 °F (20 °C), despite the fact that the ultraviolet radiation was sufficiently intense to kill all of the spores in a sample.

The crystal tends to be relatively insoluble in most solvents and under most environmental conditions. This insolubility necessitates special techniques to dissolve the crystal without significantly altering either the structure or the toxicity of the proteins of which it is composed.

Prasad and Shethna (1976) reviewed the biochemistry of the proteinaceous compounds of the crystal. They reported that a large number of dicarboxylic acids in the crystal resulted in a low pH (pH 4.4). The presence of 0.36 percent silicon by weight resulted in both the regular crystalline structure and in the insolubility of the crystal. The intact, undissolved crystal is stable when heated, although the dissolved crystal protein is denatured by heat. Both intact and dissolved crystal protein are inactivated by protein precipitants such as trichloroacetic acid and mercuric chloride. Many organic solvents such as chloroform, methanol, and acetone, however, inactivate dissolved crystal protein but not the undissolved protein of the intact crystal.

1.2.2.4 Other Toxic Metabolites. In addition to alpha-exotoxin, beta-exotoxin, and delta-endotoxin, there are various enzymes, including lecithinases, chitinases, and proteases that have been shown to damage cells and organs of various animal species when in the presence of a suitable substrate. These enzymes are collectively also known as gamma-exotoxins, although there is no clear proof of their inherent toxicity (Heimpel 1967, in Forsberg et al. 1976). Such enzymes have not been reported in commercial B.t. formulations.

The nature of other toxic metabolites produced by B.t. has been reviewed by Forsberg et al. (1976), Luthy (1980), and Luthy and Ebersold (1981). Once B.t. has invaded host insect tissues, the bacterial cells proliferate during vegetative development and produce various exoenzymes such as phospholipase C, hemolysin, chitinases, and proteases. Phospholipase C is a potent enzyme for destroying cellular membranes. Chitinases destroy the hard exoskeleton (composed of chitin) of insect bodies and are thought to increase the overall toxicity of B.t. formulations to target organisms. In some applications of B.t., chitinase is included in the spray to increase the rate of penetration of B.t. through the gut lining and thus increase the effectiveness of the formulation.

In general, these additional substances play only a minor role, if any, in the overall toxicity of B.t. formulations to target organisms (Luthy and Ebersold 1981). Many nonpathogenic microorganisms also produce these same enzymes. However, the toxic effects of these enzymes are only expressed in combination with beta-exotoxin or delta-endotoxin, which are unique to B.t. (Luthy 1980). These enzymes are not present in the commercial formulations which contain only spores and crystals. However, as the bacterial cells proliferate in the vegetative phase, they are produced and serve to facilitate passage of bacteria into the hemocoel (body cavity) and the subsequent development of septicemia (infection).

Several other toxic entities associated with B.t. have been reported in the literature. Edlund et al. (1976, in Siden et al. 1979) and Siden et al. (1979) reported that B.t. var. alesti (serotype 3a) produces several proteinaceous extracellular compounds that inhibit the immune system of pupae of Saturnid moths. One of these immune system inhibitors was shown to be toxic to the fruit fly, (Drosophila melanogaster) when injected intraabdominally into adults (Siden et al. 1979). Several publications (Dulmage et al. 1981, Dulmage and Beegle 1982) refer to a "louse-factor," a substance that is toxic to mammal-biting lice and is found in formulations containing the spore-endotoxin complex of B.t. var. kurstaki.

1.2.3 Effects of Other Pesticides on Bacillus thuringiensis Formulations

Several studies have addressed the potential for interaction between B.t. and other pesticides or adjuvants that could either be applied to the same areas as the B.t. formulations or be applied together with such formulations. In some cases, the application of other pesticides with B.t. has resulted in inhibition of activity of B.t. formulations.

Morris (1981) reported on the effects of chemical additives and of the insecticide acephate on the biology of B.t. Germination of spores of B.t. var. kurstaki was significantly inhibited by chemical additives (SBP-1382 and Gardona) at a concentration of 1 ppm. Acephate, a chemical insecticide used for control of the same species as B.t., had no effect on the bacteria at a concentration of 10,000 ppm, however. In an earlier study, Morris (1975) showed that replication of B.t. cultured from Thuricide 16B was inhibited by fenitrothion at 2 ppm active ingredient (a.i.) and by a synthetic pyrethroid SBP-1382 and by gardona at concentrations of 1 ppm a.i.

Chen et al. (1974) tested the effects of seven organophosphate and carbamate insecticides (stirophos, phosmet, trichlorfon, propoxur, carbaryl, carbofuran, and methomyl) on two *B.t.* formulations, Biotrol BTB183-25W (2,500 IU/gram), which was formulated from a strain that does produce the beta-exotoxin, and Biotrol XK (6,500 IU/gram), which was formulated from a *B.t.* strain that does not produce the beta-exotoxin. They reported that carbaryl and stirphos decreased the viability of spores from the Biotrol XK formulation but did not affect the spores of Biotrol BTB. The other five insecticides had no effect on either formulation of *B.t.*

Stimulation of germination of *B.t.* spores has also been observed. Habib and Garcia (1981) reported that methyl parathion at 1.88 and at 3.75 ppm stimulated germination and reproduction of *B.t.* var. *kurstaki*. An experimental pyrethroid formulation at 1.44 ppm (pH 6.5) and at 2.89 ppm (pH 8.6) also stimulated germination of spores of this variety of *B.t.*

2.0 Toxicity of *Bacillus thuringiensis*

The effects of the various toxic entities of B.t. and its formulations have been studied in a wide variety of organisms, including target insects, nontarget insects, fish, birds, and mammals. Many of these studies were summarized by Forsberg et al. (1976). The appendices of this pesticide background statement describe the studies on the toxicity of B.t. formulations.

A majority of the studies that address the potential toxic effects of B.t. and/or its formulations do not specify the toxin that was actually associated with the observed effect, or they do not specifiy that the observed effect was due solely to the delta-endotoxin without interference from other toxins produced by B.t. Other studies are specifically concerned with the effects of toxins other than the delta-endotoxin, particularly the beta-exotoxin. For these reasons, there is very little information available on the specific toxicological effects of the delta-endotoxin on nontarget organisms at any phyletic level.

Since formulations used for USDA Forest Service applications are free of the beta-exotoxin, and since the manufacture of formulations derived from B.t. destroys any heat-labile alpha-exotoxin that might have been formed by vegetative cells during commercial production of these pesticides, this pesticide background statement is concerned primarily with the potential toxic effects of the delta-endotoxin rather than with the other toxic entities for nonmammalian species. Some data are presented for other toxic components of B.t. formulations, particularly in mammals, to emphasize the differences in toxic effects between formulations used by the USDA Forest Service and other formulations, because the alpha-exotoxin is produced during the vegetative phase, and because the exact toxins involved are not clearly defined in some formulations studied in the literature. Table 2-1 summarizes toxicity data for B.t.

The mode of action of the toxic constituents of formulations derived from B.t. has been discussed by several authors, including Bulla et al. (1975), Cherwonogrodzky (1980), Fast (1981), Fast and Donaghue (1971), Faust et al. (1974), Sebesta et al. (1981), Subramanyam and Cutkomp (1985), Thomas and Ellar (1983). Cherwonogrodzky (1980) summarized the etiology of infection by B.t. in insects as follows. The insect first ingests plant material contaminated with crystalline protoxins (the delta-endotoxins) and spores of B.t. The crystals dissolve in the alkaline environment of the insect gut, releasing proteins. The proteins are then degraded to polypeptide fragments by the proteolytic enzymes produced in the insect gut. The release of the polypeptide fragments in turn alters the

Table 2-1

Summary of the Toxicological Properties of *Bacillus thuringiensis*[a]

1. Acute Oral Toxicity

Organism (Formulation)	Exposure	Adverse Effects
Mouse (*B. thuringiensis*) (? beta-exotoxin)[b]	Administered in water at 0.3 and 15 x 10^6 IU/kg.	No deaths reported after 7 days observation.
Mouse (Thuringiensin) (beta-exotoxin)	Administered for 8 days.	No LD_{50} observed at 200-μg/g dose.
Mouse (Thuricide) (beta-exotoxin)	Administered at 15% in feed for 7 days.	No gross pathologies. Cells of *B. thuringiensis* were isolated from tissues.
Hog (Thuricide) (beta-exotoxin)	Administered 2.7 x 10^{13} spores daily for 5 days.	No pathological effects or effects on weight gain.
Rat (Thuricide) (beta-exotoxin)	Administered up to 24 g/kg (2 x 10^{12} viable spores).	No deaths or signs of toxicity. No gross or histological pathologies.
Human (Biotrol) (? beta-exotoxin)	Administered 1 g (10 x 10^9 spores) in diet for 3 days.	Physical, hematological, and clinical tests were normal.
Human (Thuricide) (beta-exotoxin)	Administered 1 g (3 x 10^9 spores) by capsule for 5 days.	No adverse effects observed.
Mouse (Biotrol) (? beta-exotoxin)	Administered up to 8% in feed (10^{10} spores/g).	No toxic effects reported after 11 days.
Rat (Biotrol) (? beta-exotoxin)	Administered in feed at up to 10 g/kg body weight for 1 day.	No deaths observed after 13 days. No effects reported on weight gain or blood counts.
Cattle (Biotrol) (? beta-exotoxin)	Administered 2.7 x 10^{13}/day.	No effects reported.
Dog (Dipel) (? beta-exotoxin)	Administered up to 6 x 10^6 IU/kg body weight.	No deaths reported after 48 hrs.

Table 2-1 (Continued)

2. Acute Parenteral Toxicity

Organism (Formulation)	Exposure	Adverse Effects
Mouse (Thuricide)	Intraperitoneal injection of 3 x 10^8 viable organisms. Test material washed free of carrier material.	No deaths or abnormal symptoms were observed.
Mouse (Thuricide)	Intraperitoneal injection of 3 x 10^8 nonviable organisms. Test material autoclaved but contained carrier material.	All mice died within 16 hrs.
Mouse (broth culture) (alpha-endotoxin)	Intraperitoneal injection of 2 ml of broth culture.	All mice died within 24 hrs.
Mouse (purified beta-exotoxin)	Intraperitoneal injection of 0.2 ml of exotoxin, specific dose not stated.	LD$_{50}$ = 18 µg/g body weight. Mice died on third day after injection.
Mouse (*B.t.* var. *thuringiensis* beta-exotoxin).	Intraperitoneal injection of 0.5 ml beta-exotoxin at doses of 100 to 800 µg.	LD$_{50}$ = 13.3 µg/g body weight. Hepatic necrosis was observed at autopsy.
Mouse (*B.t.* var. *thuringiensis* beta-exotoxin)	Subcutaneous injection of 0.5 ml beta-exotoxin at doses of 100 to 800 µg.	LD$_{50}$ = 16.6 µg/g body weight. Hepatic necrosis was observed at autopsy.
Guinea Pig (Thuricide)	Intraperitoneal injection with 4 ml of a 24-hr-old culture.	No deaths after 7 days.
Guinea Pig (Thuricide)	Intraperitoneal injection 1 ml washings containing relatively high bacterial concentrations.	Seven of 10 animals died during a 7-day observation period.
Guinea Pig (Biotrol)	Intraperitoneal injection of 5 x 10^8 spores.	No deaths after 14 days. *B.t.* detected in tissues.
Rabbit (Biotrol)	Intraperitoneal injection of 1 x 10^9 spores.	No deaths after 14 days. *B.t.* detected in tissues.
Hog (Biotrol)	Intraperitoneal injection of 2 x 10^9 spores.	No deaths after 4 days. *B.t.* detected in tissues.

Table 2-1 (Continued)

3. Acute Dermal Irritation

Organism (Formulation)	Exposure	Adverse Effects
Rabbit (Dipel)	Applied on shaved and on shaved and abraded skin.	No irritation after 3 days.
Rat (Thuricide 32B)	Abraded and nonabraded skin to distilled water control and 13.2 ml/kg Thuricide 32B	Thuricide 32B is not a skin irritant. No apparent infectivity of *B.t.* in rats 14 days after dermal application. (Sandoz unpublished 1981).

4. Acute Inhalation

Organism (Formulation)	Exposure	Adverse Effects
Mouse (Thuricide) (beta-exotoxin)	Inhalation of powdered material during 4 exposures of 15 mins. each over a 6-day period.	No toxic effects were observed.
Mouse, Rat, and Guinea Pig (Dipel)	Inhalation of aerosol, 5-10 animals/group, 20% suspension for 10 mins.	No deaths 1 wk following.

5. Acute Ingestion and Inhalation

Organism (Formulation)	Exposure	Adverse Effects
Human (Thuricide) (beta-exotoxin)	Ingestion of 1 g (3×10^9 spores) and inhalation of 100 mg test material daily for 5 days.	No adverse effects were observed.

6. Acute Eye Irritation

Organism (Formulation)	Exposure	Adverse Effects
Rabbit (Dipel)	Ocular instillation of 0.1 ml of a 20% suspension.	No irritation was observed.
Human (Dipel)	Accidental exposure of one eye. Eye immediately washed and treated with antibiotic.	Eye became irritated. Corneal ulceration reported after 10 days. *B.t.* was isolated from the lesion. Eye healed after 2 wks of topical treatment.

Table 2-1 (Continued)

7. Subchronic Oral Toxicity (Cont'd)

Organism (Formulation)	Exposure	Adverse Effects
Rat (Biotrol)	Administered 0, 1.25, 2.5, 5.0, and 10.0% in diet for 49 days.	No differences were observed between test and control animals.
Sheep (Thuricide HP and Dipel)	Dietary .500 mg/kg/day Thuricide HP and Dipel for 5 mos.	No significant compound-related toxicity were detected when treated sheep were compared to control sheep under identical conditions. *B.t.* was essentially an avirulent organism for sheep. (Sandoz unpublished 1981).
Rat (Thuricide)	Oral gavage of $1^{-3} \times 10^{7}$, $1^{-3} \times 10^{8}$, and $1^{-3} \times 10^{9}$ spores/kg of Thuricide for 13 wks.	No systematic toxicity or evidence of infectivity from Thuricide was observed. (Sandoz unpublished 1981).

8. Dermal Sensitization

Organism (Formulation)	Exposure	Adverse Effects
Guinea Pig (Thuricide) (Beta-exotoxin)	Intracutaneous injection of 10 doses of 0.1% suspension. First injection of 0.05 ml, remaining injections of 0.1 ml each every other day for 3 wks.	No evidence of dermal sensitization reported.
Guinea Pig (Thuricide) (beta-exotoxin)	Topical application of powder to abraded skin every other day for 3 wks. First application of 25 mg, remaining 9 applications of 50 mg each.	Local irritation observed, but no evidence of any allergic response.
Guinea Pig (Thuricide) (beta-exotoxin)	Topical application of powder to intact skin every other day for 3 wks. First application of 25 mg, remaining 9 applications of 50 mg each.	No evidence of any allergic response was reported.

Table 2-1 (Continued)

8. Dermal Sensitization (cont'd)

Organism (Formulation)	Exposure	Adverse Effects
Guinea Pig (*B.t.* in Tween 20 or in Prophyllite dust)	Sensitization by simultaneous subcutaneous and intramuscular injection of a total dose of 0, 0.4, 8, 40, 200, or 400 mg test material. Challenge occur occurred 21 days later.	Deaths (6/6, 6/6, and 2/6 at dose levels of 400, 200, and 40 mg, respectively) occurred within 18 to 24 hrs of the sensitizing dose. No deaths were observed among the remaining animals.

9. Chronic Toxicity

Organism (Formulation)	Exposure	Adverse Effects
Rat (Biotrol)	Oral administration for 2 years of 25 x 10^9 spores/g diet at a concentration of 1%.	No differences were observed between test and control animals
Human (Commercial material)	Exposed for up to 7 mos to whole fermentation broth, moist bacterial cake, effluent, and final powder.	No adverse effects were reported.

10. Mutagenicity

Organism (Formulation)	Exposure	Adverse Effects
Salmonella typhimurium (*B.t.* var. *morresoni*)	Bacterial strains TA1535, TA1537, TA1538, TA98, and TA100 exposed to varying concentrations (1,000 to 100,000 µl/plate) of exotoxins (autoclaved supernatant) with or without rat liver S9.	Not mutagenic. No significant increase in revertant colonies.
Saccharaomyces cerevesiae (*B.thuringiensis* serotypes 1 and 2, and purified beta exotoxin)	Cells treated with autoclaved supernatant of serotypes 1 and 2, and with purifiedexotoxin.	Not mutagenic. No induction of mitotic gene conversion.
Human lymphocytes (*B.t.*, serotype 1; beta-exotoxin)	Cultures treated with 20% v/v concentration of autoclaved supernatant.	Increased incidence of chromosome aberrations was reported.

Table 2-1 (Concluded)

10. Mutagenicity (cont'd)

Organism (Formulation)	Exposure	Adverse Effects
Human lymphocytes (*B.t.*, serotype 3; no beta-exotoxin)	Cultures treated with 20% v/v concentration of autoclaved supernatant.	No increased incidence of chromosome aberrations was reported.
Drosophila melanogaster (*B.t.*, serotypes 1 and 2, and purified beta-exotoxin)	Autoclaved supernatant was administered in diet during entire period of development.	Nondisjunction tests were negative. Slight but significant ($p \leq 0.01$) increase observed in sex-linked recessive lethal mutants in animals exposed to supernatant from serotype 1.
Allium cepa, *A. sativum*, and *Vicia faba* (beta-exotoxin and delta-endotoxin)	Growing root meristems were exposed to 50 ppm solutions of each for 1 to 3 hrs.	The beta-exotoxin caused clastogenesis only in *A. sativum*. Major aberrations were chromosome breaks, gaps, and occasional chromatid gaps.
Rat (*B.t.*, serotype 1; beta-exotoxin).	Autoclaved supernatant concentrations of 100%, 50%, and 5% were administered in the drinking water for 3 mos.	No increased incidence of chromosomal aberrations in bone marrow of animals administered up to 100%. Increased incidences of chromosomal aberrations in lymphocytes of animals fed 50% and 100% supernatant.
Rat (*B.t.*, serotype 1; beta-exotoxin)	Autoclaved supernatant at a concentration of 100% (v/v) administered in drinking water for 6 mos.	No increase in chromosome aberrations compared to controls in bone marrow.
Rat (*B.t.*, serotype 1; beta-exotoxin)	Lethal dose administered by intraperitoneal injection.	No induction of chromosome aberrations in bone marrow.
Rat (*B.t.*, serotype 1; beta-exotoxin)	Autoclaved supernatant at a concentration of 50 mg/kg body weight was administered hourly for 9 hrs. The last injection was a sublethal dose.	Not mutagenic. No increase in sister chromatid exchanges compared to controls for bone marrow.

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[a] References can be found in the Appendices.

[b] "?" indicates that formulation as tested may have contained the specified toxin.

permeability of the insect gut epithelial cells, which burst, resulting in a distortion of the gut wall. Subsequently, the pH of the gut is reduced and the *B*.*t*. spores germinate. The vegetative bacterial cells then invade the hemocoel of the insect where they rapidly multiply, destroy tissues, and finally kill the infected insect. These vegetative cells are then passed through the food chain when the infected insect is eaten by other insects, or the insect dies and releases bacteria to continue the cycle of infection. Other insects feeding upon the infected insects do not appear to be harmed via the food chain (Yousten 1973). Similarly, oral laboratory doses and field studies indicate that *B*.*t*. does not harm bird predators who eat those insects (Abbott 1985, and Buckner et al. 1974) (see section 2.4).

Specific toxicological effects in target insects, summarized by Bulla et al. (1975), include effects of the delta-endotoxin associated with the parasporal crystal body, as well as effects of the beta-exotoxin. These effects include paralysis of the gut and/or entire body, increased gut secretory activity, detachment of the gut cells from the basement membrane of the intestine, increased permeability of the gut wall to sodium ions, slower rate of glucose uptake into the hemolymph, increased potassium ion concentration in the hemolymph, and blockage of nerve conduction. The beta-exotoxin has been shown to compete with ATP (adenosine triphosphate) for the active site of RNA polymerase. It may also inhibit other enzymatically catalyzed reactions.

Formulations derived from *B*.*t*. are used primarily to control insect pests on a variety of agricultural crops as well as on forest and ornamental trees. Most studies on the toxicity of *B*.*t*. and its formulations are concerned with their efficacy in controlling a variety of actual or potential target invertebrate pests, usually lepidopteran larvae. The results of some of these studies, in which the delta-endotoxin was clearly indicated to be the toxic entity involved, are briefly summarized in table 2-2 and will not be considered further in this pesticide background statement.

The ultimate purpose of mutagenicity studies is to determine the potential mutagenic effects of the test compound in humans. Therefore, in the following discussion of the toxicity of *B*.*t*. and of formulations derived from *B*.*t*., mutagenicity studies are discussed in the section on mammalian toxicity regardless of the specific test system or organism used.

2.1 Microorganisms

No studies on microorganisms were identified in the literature dealing with the potential toxic effects of *B*.*t*. formulations

Table 2-2

Effects of *Bacillus thuringiensis* on Target Organisms[a]

Organism	Formulation	Effects	Reference
Lepidoptera			
Bollworm, *Earias insulana*	Thuricide HP (3.2% WP *B.t.*; 16,000 IU/mg; 7.3 BIU/lb).	Weekly treatments for 8 wks at 0.5 or 1.0 lb/acre (3.6 to 7.3 BIU/acre) did not control infestation in cotton. Treatment with *B.t.* at 0.5 lb/acre (3.6 lb/acre) plus azinphos methyl at 0.5 lb/acre (3.6 BIU/acre) not significantly different from treatment with azinphos methyl alone.	Hussain and Askari 1976
Tobacco Budworm/ Bollworm Complex, *Heliothis virescens* and *H. zea*	*B.t.* strain HD-1 wettable powder (5.4 BIU/lb; 11,900 IU/mg) and dust (2.2 BIU/lb; 4,800 IU/mg).	Applications of 63 BIU/acre (dust) or 43 BIU/acre (wettable powder) for 13 or 8 applications, respectively, effectively controlled both tobacco budworm and bollworm.	McGarr et al. 1970
Tobacco Budworm, *Heliothis virescens*	*B.t.* strain HD-1 wettable powder (16,000 IU/mg; 7.3 BIU/lb).	Applied at 560 g/ha (5 lb/acre; 3.63 BIU/acre alone, with feeding adjuvant, with nuclear polyhedrosis virus (NPV), and with both NPV and adjuvant. All resulted in control of budworm, with greater control in presence of adjuvant.	Bell and Romine 1978

Table 2-2 (Continued)

Organism	Formulation	Effects	Reference
Tobacco budworm, *Heliothis virescens*	*B.t.* strain HD-1, specific activity not given.	Toxicity of the delta-endotoxin-spore complex given as LC_{50} (95% confidence limits) in IU/ml diet: - 7 days' survival: 69.52 (89.63-56.41) - to 3rd instar: 23.98 (26.30-21.90) - to pupation: 10.87 (12.12-9.70) - to emergence: 4.56 (5.09-4.04). Sublethal amounts of *B.t.* in diet resulted in increased development time, decreased weights of surviving pupae, and reduced numbers of individuals that reached adulthood as concentrations increased.	Dulmage and Martinez 1973
Cotton Bollworms, *Heliothis spp.* and Rough Bollworm, *Earias hueglei*	Dipel, *B.t.* var. *kurstaki*, (16,000 IU/mg; 7.3 BIU/lb).	*B.t.* less effective in the field than formamide insecticides (chlordimeform and amitraz). No synergism observed between *B.t.* and chemical insecticides.	Wilson 1981
Indian meal moth, *Plodia interpunctella*	Dipel, *B.t.* var. *kurstaki*, (16,000 IU/mg; 7.3 BIU/lb).	Moths fed *B.t.* in diet developed a nearly 30-fold increase in resistance in 2 generations and plateau of 100-fold resistance after 15 generations. Resistance was stable with discontinuance of exposure, was inherited as a recessive trait, and was also observed in field populations of the same species collected in grain bins.	McGaughey 1985

Table 2-2 (Continued)

Organism	Formulation	Effects	Reference
European Skipper, *Thymelicus lineola*	Dipel WP, Dipel SC (45B) Thuricide HPC, Thuricide 26B, and Novabac 3, specific activity not given.	Field application at rates ranging from 2.5 to 10 BIU/ha (1.01 to 4.04 BIU/acre) resulted in decreased mean larval density to 0.1 or less that of control plots sampled 11 or 13 days after application. Also significantly less defoliation of timothy crops was reported. Mortality of skipper occurred less rapidly with *B.t.* formulations than with chemical insecticides, however.	Letendre and McNeil 1979
Forest Tent Caterpillar *Malacosoma distria*	Dipel WP, Dipel LC, Dipel SC, Thuricide 16B; specific activity not given.	Heavy larval mortality and effective foliage protection (prevention of 20% net foliage loss) obtained with 8.6 BIU/ha (3.4 BIU/acre) of Dipel WP; 4.8 and 9.6 BIU/ha (1.94 and 3.89 BIU/acre of Dipel LC; and 9.6 BIU/ha (3.89 BIU/acre) of Thuricide 16B. Dipel SC, applied at 9.6 BIU/ha (3.89 BIU/acre), did not control caterpillar populations.	Harper and Abrahamson 1979
Gypsy Moth, *Lymantria dispar*	Dipel, specific activity not given.	Applied at 20 BIU/ha; 6 liters per 0.4 ha (8.1 BIU/acre; 1.6 gal/acre) twice by aerial spray to the 2nd and 3rd instar larvae 1 wk apart. Ninety-nine percent reduction in gypsy moth populations was observed in treated plots. Average defoliation in treated areas was 29%.	Reardon et al. 1979

Table 2-2 (Continued)

Organism	Formulation	Effects	Reference
Spruce budworm, *Choristoneura fumiferana*	Dipel WP, 16,000 IU/mg; 7.3 BIU/lb or Thuricide, 4.2 BIU/l; 15.9 BIU/gal. Uniform comparison of units was not provided.	Applied in the field with and without either fenitrothion or Orthene to white spruce and balsam fir trees. Emergence of adults was substantially reduced by 20 to 30 BIU/ha (8.1 to 12.15 BIU/acre) *B.t.* + Orthene treatments. Both Thuricide and Dipel in combination with fenitrothion, as well as 30 BIU/ha (12.15 BIU/acre) Dipel + Orthene, resulted in reduced oviposition. Parasitism of eggs was reduced with treatment by *B.t.* + low doses of fenitrothion, but *B.t.* + low doses of Orthene had no major effect on parasites of eggs, pupae, or larvae.	Morris and Armstrong 1974
Spruce Budworm, *Choristoneura fumiferana*	Novabac 32B, Dipel 36B, and Thuricide 32B, specific activity not given.	*B.t.* formulations applied in the field to balsam fir with either sorbitol or sodium dihydrogen phosphate for 2 yrs. Final larval mortality exceeded 80% in 84.5% of the treatments.	Smirnoff 1979a
Spruce Budworm, *Choristoneura fumiferana*	*B.t.*, serotype 3a, 3b, specific activity not given.	Surviving pupae from treated areas were considerably less vigorous than were pupae from untreated areas. *B.t.* had detrimental effects on survivors at sublethal exposures.	Smirnoff 1983

Table 2-2 (Continued)

Organism	Formulation	Effects	Reference
Spruce Budworm, *Choristoneura fumiferana*	Dipel WP (3.2%), 16,000 IU/mg; 7.3 BIU/lb (at least 25 x 10^9 viable spores/mg); Thuricide HP 16B, 3,300 IU/mg; 1.5 BIU/lb (at least 5 x 10^6 viable spores/mg).	Fifth instar larvae tested with both formulations, with and without chitinase. Chitinase increased the efficacy of Thuricide at all doses. Dipel was more effective than Thuricide with chitinase at most doses. Addition of chitinase to Dipel did not increase the efficacy of Dipel at any dose level, however.	Nigam 1974
Spruce Budworm, *Choristoneura fumiferana*	Thuricide 16B, specific activity not given.	Aerial treatments included: *B.t.* alone; *B.t.* + chitinase; and *B.t.* + Orthene. All treatments provided satisfactory protection of foliage with no statistical differences between treatments. No significantly better protection observed with *B.t.* + Orthene compared to treatments.	Dimond and Spies 1980
Spruce Budworm, *Choristoneura fumiferana*	Thuricide 16B, Dipel 4L 32B, and Dipel 4L 24B, specific activity not given.	All formulations applied at 8 BIU/acre (19.75 BIU/ha) in a total volume of 1/4 to 1 gal/acre with varying dilutions from 0.5 to 75% water. Treatments did not produce detectable population reduction but did provide significant foliage protection (except Thuricide applied at 1 gpa). Total amounts of foliage saved were insubstantial due to a high number of worms and cool and wet weather.	Dimond et al. 1981

Table 2-2 (Continued)

Organism	Formulation	Effects	Reference
Diamondback Moth *Plutella xylostella*	*B.t.* var. *kurstaki*, (Thuricide HP 3.2%), specific activity not given.	Bioassay based on mortality of larvae on cabbage treated with *B.t.* Mortality, determined from a regression line, was 100% among larvae exposed to leaves immediately after treatment and 50% among larvae exposed to leaves 6 days after treatment.	Mohamad et al. 1980
Diamondback Moth *Plutella xylostella*	*B.t.* var. *kurstaki* (Thuricide HP 3.2%), specific activity not given.	Application of 450 g in 450 ℓ of water/ha (0.4 lb in 48 gal water/acre) resulted in significant ($p \leq 0.05$) reduction in both larval population and in damage to cabbages in field tests.	Mohamad et al.(1979)
Cabbage Looper, *Trichoplusia ni*	*B.t.* strain HD-1: 9,900 UI/mg; (4.5 BIU/lb).	Formulations were effective in field tests when 1.1 to 4.5 BIU/acre (2.72 to 11.11 BIU/ha were applied once a week during the growing season. Application rates of 2.2 to 4.5 BIU/acre (5.44 to 11.11 BIU/ha) were effective when applied once a week or once every 10 days.	Dulmage et al. 1971
Diptera			
Mosquito, *Aedes aegypti* (larvae)	*B.t.* var. *kurstaki*, HD-1, serotype 3a, 3b derived from Dipel and *B.t.* var. *thuringiensis*, BA-068, serotype 1, specific activity not given.	Of six *B.t.* strains tested, two were toxic to larvae after 24 hrs, exposure. LC_{50}'s were 5.6×10^4 and 2.4×10^5 spores/ml for HD-1 and for BA-068, respectively. The toxic entity was heat sensitive, gamma-ray resistant, and appeared to be associated with the crystal body.	Panbangred et al. 1979

Table 2-2 (Continued)

Organism	Formulation	Effects	Reference
Mosquitoes, *Aedes triseriatus* and *Culex tarsalis*	*B.t.* var. *kurstaki*, serotype 3a, 3b, 15 strains tested, specific activity not given.	LC_{50}'s were reported to range from 0.04 to 2.0 μg/ml after 7 days, exposure.	Hall et al. 1977
Mosquitoes, *Aedes aegypti*, *Anopheles dirus*, and *Culex quinquefasciatus*	*B.t.* strain HD-1, specific activity not given.	LC_{50}'s were 1.2×10^5, 1.1×10^4, and 8.2×10^5 bacterial cells/ml, respectively, for the three species of mosquito. Toxicity was due to parasporal material. No toxicity was observed among larvae of *Toxorhynchites splendens*, a fruit and vegetable feeding mosquito, the larvae of which prey on the larvae of other mosquito species.	Samasanti et al. 1982
Mosquito, *Adedes aegypti*, (larvae and adult females)	*B.t.* var. *kurstaki*, and *B.t.* var *thuringiensis*, solubilized and intact crystals from both varieties, specific activity not given.	Preparations from *B.t.* var. *kurstaki* had no lethal effect on larval or adult mosquitoes. LC_{50}'s reported as >75 and >50 μg/ml among larvae exposed for 24 hrs to solubilized and intact crystal material, respectively. LC_{50}'s reported as >0.54 and >2.16 μg/ml of adult wet weight after exposure for 48 hrs to solubilized and intact crystal material, respectively, administered by enema to adult females. Crystals of *B.t.* var. *thuringiensis* were lethal, with reported 24-hr LC_{50}'s of 1.28 and 0.018 μg/ml among larvae exposed to solubilized and intact crystal material, respectively and 48-hr LD_{50}'s of 0.04 and 0.21 μg/mg mosquito among adult females exposed to solubilized and intact crystal material, respectively.	Klowden et al. 1983

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Table 2-2 (Concluded)

Organism	Formulation	Effects	Reference
Mosquito, *Culex tarsus*	*B.t.* strain HD-1, specific activity not given.	Effective against larvae in laboratory tests. Its toxic activity to early instars was approximately 10^6 spores/ml. Delayed pupation and reduced emergence of viable adults were reported.	Goldberg and Ford 1974
Blackflies, *Simulium spp*.	*B.t.* var. *kurstaki*, strain HD-255, serotype 3a, 3b, specific activity not given.	Six species of *Simulium* were tested for toxicity. The mean mortalities for a 24-hr exposure period after 1 wk of observation were: 5 to 85% with exposure to 1.0 ppm; 13 to 97% with exposure to 10.0 ppm; and 3 to 11% for controls For *S. vittatum* exposed for 24 hrs to 0.05 ppm, the mean mortality for exposure to crystal was 67%; to spores, 17%. Mortality among controls was 13%.	Lacey et al. 1978

[a]Additional studies are discussed in section 1.2 on use of *B.t.* formulations.

containing only the delta-endotoxin as the toxic entity. For this reason, there are no studies listed in appendix A that summarize toxic effects of *B.t.* or its formulations on other microorganisms.

2.2 Invertebrates

Relatively few studies have been conducted among nontarget invertebrate organisms using formulations that contained only the delta-endotoxin as the specified toxic agent of *B.t.* The available studies were primarily concerned with potential effects on beneficial organisms that could be directly exposed to *B.t.*, or on organisms that could bioaccumulate amounts of *B.t.* or its toxins, such as honey bees, insect parasites and parasitoids, and earthworms. For the most part, these studies, described in appendix B, indicate that use of *B.t.* formulations at application rates that are generally utilized does not appear to have any adverse effects on nontarget invertebrate species that are exposed to the bacterium. However, more data on the toxic effects of the delta-endotoxin, particularly from *B.t.* var. *kurstaki*, under actual field conditions are needed to assure that the toxic effects of this strain to nontarget invertebrates are indeed low. It is particularly important that these studies quantify the amounts of delta-endotoxin to which the test animals are exposed. In addition, field studies on the potential for accumulation of the alpha-exotoxin produced by vegetative cells should be conducted in invertebrates as well as in their vertebrate predators.

Several studies on the effects of *B.t.* formulations on honey bees were reviewed by Forsberg et al. (1976). In only one of these studies (Cantwell et al. 1966, in Forsberg et al. 1976) was the toxic entity specifically identified as the delta-endotoxin. Delta-endotoxin derived from three varieties of *B.t.* (var. *thuringiensis*, *alesti*, and *sotto*) was fed to brood and adult honey bees (*Apis mellifera*) in the diet. No detectable mortality or other ill effects were noted among bees fed 0.5 to 16 x 10^6, 0.5 x 10^6, or 0.25 to 0.50 x 10^6 per bee (units not given but assumed to be spores on the basis of context) of delta-endotoxin derived from *B.t.* (var. *thuringiensis*, *alesti*, and *sotto*), respectively.

The effects of *B.t.* formulations have been studied primarily in parasites and in parasitoid wasps (parasite-like wasps that lay their eggs in paralyzed insect prey) that are predators of the lepidopteran species that are the actual targets of *B.t.* application. Most of the studies reported no adverse effects following pesticide application at normal rates in the field for control of lepidopteran pests. One study, however, reported significantly fewer adults of several species of tachinid and sarcophagid flies and of braconid and chalcid wasps in treated plots compared to controls (Reardon et al. 1979). Plots were sampled both during the year of treatment and 1 year after treatment, following two applications of 8.1 BIU Dipel/acre (20 BIU/ha) by aerial spray. In this study, the specific activity of the formulation was not provided and the authors did not specify the variety of *B.t.*, or its serotype, from which the formulation was derived. Elsey (1973) reported that Dipel (*B.t.* var. *alesti*, HD-1, 5 percent wettable powder) did not reduce numbers of the predatory spined stiltbug when applied twice to tobacco at a 5-week interval and at a rate of 5 lb/acre (5.6 kg/ha). The specific activity of the formulation was not indicated.

Early applications of *B.t.* in the field were generally at rates of 8 BIU/acre (20 BIU/ha) at two application dates to control gypsy moths (*Lymantria dispar*). Andreadis et al. (1983) reported successful results from use of Dipel 4L at two application rates 12 and 16 BIU/acre (0.4 ha) by helicopter only once. A single application of 12 and 16 BIU/acre gave significant reductions of gypsy moth larvae. In the treated plots, there was 4 percent net defoliation, in untreated control plots, 69 percent defoliation. *B.t.* also was reported to be compatible with major natural enemies.

Several studies have been conducted with insects under laboratory conditions. Wilkinson et al. (1975) exposed various life stages of insect parasitoids and predators, for as long as 120 hours, to Thuricide HPC (specific activity not given and variety of *B.t.* or serotype not indicated but presumed to be HD-1). The insects were exposed on dried filter paper previously treated with *B.t.* at a rate equivalent to 16.2 BIU/acre (40 BIU/ha). No mortalities were reported among adults of the wasp or fly genera tested, or among larvae of the lacewing genus tested. Adult beetles and adult lacewings both showed 3.3 percent mortality. In a bioassay with Thuricide (4,000 IU/mg, serotype not given but presumed to be HD-1), Tipping and Burbutis (1983) exposed parasitic wasps to pepper plant leaves that had been treated with *B.t.* at a rate equivalent to 3.97 BIU/acre (9.8 BIU/ha) and then allowed to weather for as long as 21 days. They reported no effects on the wasps in terms of reduced rates of emergence from, or parasitism of, European corn borer eggs. The toxic effects of a diet consisting of prey insects infected with *B.t.* were examined in a study reported by Yousten (1973). Chinese praying mantids were fed cabbage looper larvae that had ingested a lethal dose of *B.t.* var. *kurstaki* (specific activity of 18,000 IU/mg). The mantids received a diet of infested looper larvae for 5 days and were then observed for an additional 7 days while feeding on a diet of larvae that were free of *B.t.* infestation. No mortality or abnormal behavior was observed among the mantids.

The potential effect of B.t. formulations on earthworms was investigated by Benz and Altwegg (1975). Dipel (16,000 IU/mg, serotype H3), applied to forest soil in the field at rates of 0.536, 5.36, or 53.6 lb/acre (60, 600, or 6,000 mg/m^2), had no adverse effect on earthworm populations (no species identified) over a period of up to 9 weeks.

2.3 Fish

No data are available on the toxic effect on fish of B.t. formulations in which only the delta-endotoxin has been identified as the toxic entity. Studies on fish, summarized in appendix C, were pre-EPA tolerance, and therefore may have contained the beta-exotoxin. EPA (1985) has reported that the beta-exotoxin was toxic to coho salmon. It is possible the B.t. *kurstaki* variety is toxic to mussels and brine shrimp, and therefore EPA has not registered it for use in aquatic environments. However, B.t. appears to be of low toxicity to fish (1/30 as toxic as DDT). In field applications, there are no adverse fishery effects (Buckner 1974).

2.4 Birds

Several studies have been identified in which the delta-endotoxin was administered to birds. These studies, summarized in appendix D, indicate that the delta-endotoxin is practically nontoxic to birds by the oral route. However, additional studies in which the animals have been exposed to a known amount of delta-endotoxin, are needed to fully evaluate the potential toxicity of B.t. formulations to birds.

Tucker and Crabtree (1970) reported that the LD_{50} was much greater than 2,000 mg/kg body weight for mallards given an acute oral dose of Thuricide (50 x 10^9 spores/g, assumed to be delta-endotoxin from context). Couch (1973, in Forsberg et al. 1976) reported that the rate of weight gain among chicken pullets was lower in pullets fed 0.5 to 4.0 percent Dipel WP (B.t. var. *kurstaki*, specific activity not given) in the diet for 2 weeks than in controls. Although the authors speculated that the decrease in weight gain among the high-dose pullets (2 and 4 percent B.t. formulation in the diet) may have been due to food avoidance, they did not provide data to support this speculation.

Buckner et al. (1974) reported the effects of using Thuricide 16B and Dipel WP in the field on 15 families of songbirds. The aerial application rate of 2 to 4 gal/acre was used on plots with and without chitinase. This was a very high rate of application. Overall, there was no significant reduction in bird populations resulting from B.t. application.

2.5 Mammals

The toxicological properties of B.t. in mammals are summarized in table 2-1 and described in more detail in appendices E and G.

The potential health hazards from acute and chronic exposure of mammals to pesticide formulations derived from B.t. have been evaluated in laboratory animals such as mice, rats, guinea pigs, and rabbits, as well as in domesticated farm animals such as hogs and cattle. Effects resulting from accidental and volunteer exposures of humans to B.t. have also been reported. The toxicity of B.t. has been evaluated by exposing animals to vegetative bacterial cells, bacterial spores, crude bacterial suspensions and supernatant fluids, and purified toxins. Since the various strains of the bacillus produce different toxins and the products produced by a single strain may vary with the environmental conditions under which the formulation is produced, different results may be reported for apparently similar test materials. B.t., as currently used by the USDA Forest Service, is generally nontoxic to mammals, including humans, since the toxic alpha-exotoxin is denatured during the manufacturing process, and the strains used do not synthesize beta-exotoxin.

2.5.1 Acute Toxicity

Acute toxicity data have been reported for B.t. formulations administered by oral, parenteral (administered other than via the digestive tract), dermal, inhalation, and ocular routes. The data on acute toxicity in mammals are described in appendix E.

2.5.1.1 Oral. B.t. is generally nontoxic when administered orally to mammals. No deaths were reported among mice when B.t. was administered orally at 0.3 and 1.5×10^6 IU/kg (Kimura 1970, in Forsberg et al. 1976). The higher dose was reported to contain 27 to 120×10^{12} spores. The test formulation may have contained the beta-exotoxin as a toxic agent in this study.

De Barjac and Riou (1969, in Forsberg et al. 1976, and Sebesta et al. 1981) administered thuringiensin (the beta-exotoxin) orally to female mice. No LD_{50} was reached when up to 200 µg/g body weight of thuringiensin was fed consecutively for 8 days. There were no reported mortalities among animals given 100 µg/g body weight and the test animals were reported to tolerate at least 800 µg of thuringiensin.

When Thuricide (containing beta-exotoxin as a toxic agent) was administered to mice as 15 percent of their diet for 7 days, no gross pathological lesions were observed (Stewart 1961, in Forsberg et al. 1976). However, cells of B.t. were isolated from tissues of treated animals.

No pathogenicity or effect on weight gain was observed among hogs when Thuricide (containing beta-exotoxin as a toxic agent) was added to the feed at levels of 7.5×10^{11} spores/day for 4 consecutive days (Briggs 1959, in Forsberg et al. 1976).

No fatalities or other toxic effects were observed among rats dosed by gavage with up to 24 g of Thuricide, containing the beta-exotoxin/kg body weight after 1 week (Fisher and Rosner 1959). There were no gross or histological differences between the tissues of test or control animals.

Ten human volunteers (5 females and 5 males) ingested 1 g of Biotrol (10×10^9 spores) in the diet for 3 consecutive days (Long et al. undated, in Forsberg et al. 1976). Results of physical, hematological, and clinical tests were normal. B.t. cells were detected in the feces from 5 individuals in this study. The test material may have contained beta-exotoxin as a toxic agent.

Thirteen human volunteers ingested 1 g of Thuricide, containing beta-exotoxin as a toxic agent, by capsule daily for 5 days (Fisher and Rosner 1959). No adverse effects were noted in physical examinations that included laboratory analysis of blood and urine.

Six groups of 6 mice each were administered Biotrol containing 10^{10} B.t. spores/g. The test material was added to the feed at concentrations of up to 8 percent (Williams and Hernandez undated, in Forsberg et al. 1976). No toxic effects were reported for 11 days during which the test animals were observed. The test material in this study may have contained beta-exotoxin as a toxic agent.

Hernandez and McLean (undated, in Forsberg et al. 1976) administered Biotrol to rats with the feed at rates up to 10 g/kg body weight for 1 day. No deaths, effects on weight gain, or abnormalities in blood counts were observed among the treated animals during a 13-day observation period. The test material in this study may have contained beta-exotoxin as a toxic agent.

Steers were orally administered 2.7×10^{13} B.t. spores/day in a study reported by (Dunn 1960, in Forsberg et al. 1976). No adverse effects were reported. The test material may have contained beta-exotoxin as a toxic agent.

Three groups of 3 female dogs each were administered Dipel at levels containing up to 6×10^6 IU B.t./kg body weight (Kimura 1970, in Forsberg et al. 1976). The highest dose tested was equivalent to 400 mg/kg body weight. No deaths were reported following 48 hours of observation. The test material may have contained beta-exotoxin as a toxic agent.

2.5.1.2 *Parenteral*. The toxicity of several strains of *B.t.*, and of specific toxins isolated from strains of *B.t.* administered parentally has been examined on a number of species.

Lamanna and Jones (1965, in Forsberg et al. 1976) administered washed vegetative cells and spores of four strains of *B.t.* by either intraperitoneal or subcutaneous injection to mice. Specific LD_{50} (median lethal dose) is for both intraperitoneal and subcutaneous injection of both vegetative cells and of spores of *B.t.* (var. *thuringiensis*, *sotto*, *alesti*, and *entomocidus*) are given in appendix E. The reported LD_{50}'s were approximately one order of magnitude greater by subcutaneous injection than by intraperitoneal injection for each of the 4 strains.

Fisher and Rosner (1959) reported a series of studies in which 5 mice each were injected intraperitoneally with either a suspension of viable *B.t.* cells that had been washed free of carrier material with isotonic saline or with a suspension of cells that had been autoclaved prior to injection (nonviable cells). The cultures were derived from the *B.t.* formulation Thuricide. No deaths or abnormal symptoms were observed in those animals injected with viable washed *B.t.* cells, but all of the mice injected with autoclaved cells and carrier died within 16 hours after injection following symptoms of abdominal irritation and sensitivity. The authors attributed death to the carrier only, but did not specify whether or not the actual toxic agent had been produced by the *B.t.* cultures. In the same paper, these authors also reported on a study designed to test the virulence and toxic effects of *B.t.* contained in the blood of previously innoculated mice. A group of 5 mice were injected intraperitoneally with a broth culture of *B.t.* After 6 hours, blood was drawn from the treated mice and injected into a second group of mice. This process was repeated 6 times. Although the *B.t.* organisms survived the passage through to the seventh and last group of mice, there was no evidence of increased virulence of the organisms. No weight loss or toxic symptoms were observed in the test mice, although 1 mouse in the initial group died within 4 hours of injection, and all of the mice in this group exhibited malaise.

The alpha-exotoxin is comparatively toxic to mice compared to the other toxins produced by *B.t.* Alpha-exotoxin is of potential concern since it is produced during the vegetative growth phase. Krieg (1970, in Forsberg et al. 1976) reported that all of an unspecified number of mice died within 2 hours after intraperitoneal injection of 2 ml of broth culture containing the alpha-exotoxin. The animals exhibited initial symptoms of shock, including excitment followed by labored breathing, prior to death.

Groups of 15 mice each were injected intraperitoneally with 2 ml of an unspecified dose of purified beta-exotoxin derived from B.t. var. gelechiae (Sebesta et al. 1969, in Forsberg et al. 1976; Sebesta et al. 1981). The LD_{50} was 18 $\mu g/g$ body weight. Mice died in the third day following injection and, on autopsy, histological signs of pathology were observed in brain, liver, lymphatic system, and bone marrow.

LD_{50}'s of 13.3 and 16.6 $\mu g/g$ body weight were reported when mice were injected intraperitoneally and subcutaneously, respectively, with 0.5 ml of beta-exotoxin at doses of 100 to 800 μg (De Barjac and Riou 1969, in Forsberg et al. 1976). Hepatic lesions were observed at autopsy in liver tissues from both groups of treated mice.

Fisher and Rosner (1959) injected guinea pigs with cultures of B.t. derived from Thuricide. There were no deaths 7 days following injection intraperitoneally with 4 ml of a 24-hour-old beta-exotoxin producing culture. However, 7 of 10 animals died within 7 days when 1 ml of washings from glucose agar cultures, containing a relatively high concentration of bacteria, were injected intraperitoneally.

A series of experiments with B.t. spores (Biotrol) were reported in guinea pigs, rabbits, and hogs (Holland 1959, in Forsberg et al. 1976). No deaths were observed 14 days after a single intraperitoneal injection of 5×10^8 spores into guinea pigs, 14 days after a single intraperitoneal injection of 1×10^9 spores into rabbits, or 4 days after a single intraperitoneal injection of 2×10^9 spores into hogs. On autopsy, B.t. was detected in various tissues of all 3 species of test animals.

2.5.1.3 Dermal. No irritation was observed 3 days after a 20 percent suspension of Dipel was applied to either shaved or shaved and abraded skin of the rabbit (Kimura 1970, in Forsberg et al. 1976). Abbott (1986) provided results of several laboratory studies on rabbits for dermal toxicity. In one test with Vectobac 6AS the acute dermal LD_{50} was reported to be higher than 2 g/kg of body weight. Dipel 4L applied at higher rates (7.2 g/kg) to rabbits resulted in early erythema and eventual dry skin and soughing, leaving a smooth, hairless application site (Abbott 1986).

2.5.1.4 Inhalation. Thuricide was evaluated for inhalation toxicity by subjecting 10 mice to four exposures of 15 minutes each over a period of 6 days (Fisher and Rosner 1959). Ten grams of material that contained beta-exotoxin as a toxic agent were dispersed with a powder blower during each exposure in a 30 x 30 x 30 cm chamber. No toxic effects were observed, weight gains were normal, and gross pathology findings were negative. Ten animals were also exposed to autoclaved material to determine if the carrier of the pesticide had any effect on lung irritation. The results of this test were also negative.

No mortalities were observed among an unspecified number of mice or rats 7 days after they were exposed to an aerosol of a 20 percent suspension of Dipel for 10 minutes (Kimura 1970, in Forsberg et al. 1976).

2.5.1.5 Inhalation and Ingestion. Five human volunteers inhaled 100 mg of Thuricide daily for 5 days (Fisher and Rosner 1959). In addition to exposure by inhalation, the subjects ingested 1 g of Thuricide by capsule daily for 5 days. The test material contained beta-exotoxin as a toxic agent. All subjects remained well during the course of the test and no adverse effects were observed. All laboratory findings were negative. Parameters measured included weight, blood pressure, and respiration rate. Examinations included evaluations of the genitourinary, gastrointestinal, cardiorespiratory, and nervous systems.

2.5.1.6 Eye Irritation. No acute eye irritation was observed in rabbits following ocular instillation of 0.1 ml of a 20 percent suspension of Dipel (Kimura 1970, in Forsberg et al. 1976).

An 18-year-old farmer suffered eye irritation after accidentally splashing a suspension of Dipel in his right eye (Samples and Buettner 1983). The eye was immediately irrigated with water and treated with antibiotic ointment. The eye became irritated and was treated with corticosteroid ointment 3 days after exposure. The cornea became ulcerated 10 days after the accident. *B.t.* was isolated from the lesion, which healed after 2 weeks of topical treatment with Gentamicin.

Sandoz (1986) responded to this incident by pointing out the application of corticosteroid confounds the etiologic link with *B.t.* and questions the pathogenicity of the *Bacillus*. Their laboratory studies of direct application of *B.t.* to the eye resulted in no evidence of pathogenicity to the eye under properly controlled studies. Furthermore, production workers have not complained of eye infections.

2.5.2 Subchronic Toxicity

2.5.2.1 Oral. No pathological changes were observed in mice when spent culture medium was administered orally for 3 months (Carlberg 1973, in Sebesta et al. 1981). The specific toxins that may or may not have been present in the spent medium was not indicated.

Olson and Kwapien (1973, in Forsberg et al. 1976) fed Dipel in the diet of rats in a 13-week subchronic study. The test material in this study was free of beta-exotoxin. One group of 10 rats were fed 0.84 mg Dipel/kg diet daily for 1 week, then 840 mg/kg diet daily for the remainder of the study. A second group was fed 8.4 mg Dipel/kg diet daily for 1 week, then 8,400 mg/kg daily for the remainder of the study. A third group served as untreated controls. Detailed hematology, blood chemistry, and urinalysis were performed at 0, 4, and 13 weeks. Gross and histopathological examinations were performed at autopsy. No significant differences were observed between test and control animals for any of the parameters measured.

Biotrol was admistered in the diet to groups of 20 rats at dietary levels of 0, 1.25, 5, and 10 percent for 49 days (Hernandez and Muckenthaler undated, in Forsberg et al. 1976). No significant differences were observed between treated and control groups of animals.

2.5.2.2 Dermal Sensitization. Fisher and Rosner (1959) reported a series of dermal sensitization studies in which Thuricide (containing beta-exotoxin as a toxic agent) was tested in guinea pigs by intracutaneous injection, by application to abraded skin, and by application to intact skin. No evidence of dermal sensitization was observed among 8 guinea pigs when a 0.1 percent suspension of Thuricide in water was injected intracutaneously for 10 doses. The first injection was 0.05 ml, the other 9 were 0.1 ml, each given every other day for 3 weeks. The challenge dose was given 2 weeks later. Ten topical applications of Thuricide powder were administered every other day for 3 weeks to the intact or to the abraded skin of 8 guinea pigs. The first application consisted of 25 mg/animal, the remaining applications of 50 mg/animal. There was no evidence of any allergenic response on the intact skin. On the abraded skin, slight erythemia and edema occurred indicating local irritation, but there was no evidence of dermal sensitization.

Groups of 6 male guinea pigs were sensitized by simultaneous subcutaneous and intramuscular injection of B.t. suspended in Tween 20, a wetting agent, or in prophyllite dust (Kimura 1970, in Forsberg et al. 1976). The test animals received total doses of 0, 0.4, 8, 40, 200, or 400 mg of test substance. The animals were challenged by aerosolization 21 days after the last sensitizing dose and then observed for anaphylactic shock. Deaths (2 of 6, 6 of 6, and 6 of 6) at the dose levels of 40, 200, and 400 mg, respectively, occurred within 18 to 24 hours after the sensitizing doses. No deaths occurred among the surviving animals following the challenge doses.

2.5.3 Chronic Toxicity

Groups of 25 male and 25 female rats were fed Biotrol (25×10^9 spores/g) in the diet at a 1 percent level for 2 years (Barnes 1970, in Forsberg et al. 1976). No statistical differences were observed between treated and control groups of animals.

Fisher and Rosner (1959) reported the lack of chronic toxicity among 8 workers exposed to B.t. during various stages of the production of pesticidal formulations. The individuals were exposed to whole fermentation broth, moist bacterial cake, effluent, and final powder (10 g to several thousand pounds/day containing up to 15×10^9 viable spores/g). The workers were free of any complaints during the 7 months of observation. Two individuals underwent comprehensive medical examinations and showed no evidence of acute or chronic toxicity.

2.5.4 Reproductive and Teratogenic Effects

There are no reports in the available literature regarding the possible reproductive or teratogenic effects of B.t. in mammals.

2.5.5 Carcinogenicity

There are no reports in the available literature regarding the possible carcinogenic effects of B.t.

2.5.6 Neurotoxicity and Behavioral Effects

There are no reports in the available literature regarding the possible neurotoxic or behavioral effects of formulations derived from B.t. on mammals or other vertebrates. No reports are summarized in appendix F on neurotoxicity.

2.6 Mutagenicity

B.t. has been examined in a variety of microbial and other mutagenicity test systems. Generally, these studies have given negative results, indicating the nonmutagenicity of formulations derived from B.t. The results of these mutagenicity tests are summarized in table 2-1 and further described in appendix G.

Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98, and TA100 were exposed to varying concentrations, ranging from 1,000 to 100,000 µl/plate, of purified exotoxin from the autoclaved supernatant of B.t. var. *morrisoni* (Cantwell et al. 1982). The tests were performed both in the absence and presence of rat liver S-9 microsomal enzyme preparation. The test material was shown to be nonmutagenic in that there was no significant increase in the number of revertant mutant colonies.

Cells of the yeast, *Saccharomyces cerevisiae* strains D4 and D5, were treated with autoclaved supernatants of *B.t.* serotypes 1 and 3, and with purified beta-exotoxin (Linnainmaa et al. 1977). Serotype 1 produces the beta-exotoxin, serotype 3 does not. There was no indication of mutagenic activity. No induction of mitotic gene conversion was observed.

Meretoja et al. (1977) tested the potential for autoclaved supernatants of *B.t.* serotypes 1 and 3 cultures to increase the incidence of chromosomal aberrations in human lymphocyte cultures. When cultures of human lymphocytes were treated with a 20 percent v/v concentration of the autoclaved supernatant of *B.t.* serotype 1 (containing beta-exotoxin), an increased incidence of chromosome aberrations was observed. No increased incidence of chromosome aberrations was observed when the human lymphocyte cultures were treated with a 20 percent v/v concentration of autoclaved supernatant of *B.t.* serotype 3 (not containing beta-exotoxin).

Fruit flies (*Drosophila melanogaster*) were treated with the autoclaved supernatants of *B.t.* serotypes 1 and 3, as well as with purified beta-exotoxin. The test materials were fed to the fruit flies during the entire period of their development. Nondisjunction tests and sex-linked recessive tests were performed. A very slight but statistically significant ($p < 0.01$) increase in the frequency of sex-linked recessive lethal mutations were induced by autoclaved supernatant of serotype 1, only. All other tests were negative (Linnainmaa et al. 1977).

Growing root stems of *Allium cepa*, *A. sativum*, and *Vicia faba* were exposed to beta-exotoxin or delta-endotoxin protein. The beta-exotoxin caused clastogenesis in *A. sativum* only. The major aberrations observed were chromosome breaks, chromosome gaps, and occasional chromatid gaps. All other test materials were negative in all systems tested (Panda et al. 1979).

Autoclaved supernatant from *B.t.* serotype 1 (containing beta-exotoxin) was administered in the drinking water of rats for 3 months at concentrations of 100, 50, and 5 percent (Meretoja et al. 1977). Bone marrow cells and blood lymphocytes were examined. There were no increased incidences of chromosomal aberrations in the bone marrow of animals administered up to 100 percent concentration of supernatant, but there was an increased incidence of chromosome aberrations in the lymphocytes of animals administered 50 and 100 percent concentrations of the supernatant. In a similar study, when rats were administered autoclaved supernatant from B.t. serotype 1 in the drinking water at a concentration of 100 percent for 6 months, the bone marrow also showed no increased incidence of chromosome aberrations (Kahkonen et al. 1970). Lymphocytes were not examined in this latter study, however.

Intraperitoneal injection of rats with a lethal dose of beta-exotoxin, prepared from an autoclaved suspension of B.t. serotype 1, failed to induce chromosome aberrations in the bone marrow (Merotoja et al. 1977).

Kahkonen et al. (1979) injected rats intraperitoneally with the autoclaved supernatant of B.t. serotype 1 (beta-exotoxin) at a concentration of 50 mg/kg body weight hourly for 9 hours. The last injection included a sublethal dose (2.5 ml of supernatant concentrated 10 times). The rats were killed 12 hours later, and their bone marrows were examined for sister chromatid exchanges (SCE's). No significant increase in the incidence of SCE's over the controls was observed.

3.0 Environmental Fate

The spores and crystals of *B.t.* appear to be degraded in the field (i.e., decrease in viable spores) due to solar radiation, temperature, and vapor pressure deficits (Leonge et al. 1980). Vegetative cells, on the other hand, are subject to the bactericidal effects of various juices and extracts of many plants (Forsberg et al. 1976). The persistence of vegetative cells of *B.t.*, as well as of activity of the various toxic entities of *B.t.*, is strongly dependent on the specific nature of the formulation used and on various environmental parameters such as sunlight, humidity, and soil conditions. Viable spores last from a few days to weeks depending upon environmental conditions.

3.1 Bioaccumulation and Metabolism

There is very little information available concerning the behavior and fate of *B.t.* vegetative cells, specific formulations derived from *B.t.*, or various toxins produced by *B.t.*, either in plants or in animals. Of the information that is available, very little is concerned specifically with the delta-endotoxin. Forsberg et al. (1976) summarized much of the meager available information on the behavior and fate of *B.t.* in plants and animals.

3.1.1 Plants

A major concern addressed by the available information on the fate and behavior of *B.t.* in plants concerns the potential loss of insecticidal activity of the formulations due to bactericidal action of the plant fluids. Forsberg et al. (1976) have reviewed the literature on the effects of plant-derived bactericidal chemicals on *B.t.* Smirnoff (1967, in Forsberg et al. 1976) tested the effects of volatile substances from the crushed foliage of 34 species of plants. They reported that the effects on viability of the bacteria varied among the different varieties of *B.t.* and between cells and spores of each variety, with the vegetative cells generally more sensitive to the plant volatiles than were the spores.

Pinnock et al. (1971, 1974, and 1975, all in Forsberg et al. 1976) reported a series of studies on the fate of *B.t.* formulations following application to various plants. Following application of Thuricide 90TS to oak trees at an unspecified concentration, the half-life of viable spores was determined to be 3.9 days in a cool moist climate and 7.7 days in a hot dry climate. Viable spores of Biotol BTB 183 dust and Biotrol BTB 183 wettable powder, applied to oak trees in a hot dry climate, had half-lives of 22.7 days and less than 1 day, respectively. Following application of Amdal (Dipel WP was formerly marketed as Amdal *B.t.* Wettable Powder), Biotrol, and two formulations of Thuricide, a two-phase loss in viability was reported, with half-lives of 0.58 to 1.85 days for days 0 to 3 and of 1.20 to 2.66 days for days 3 to 7. When Dipel was applied to the point of runoff to 4 species of trees (California live oak, red bud, eucalyptus, and walnut), the half-lives of viable spores ranged from 0.38 to 0.90 days. Deposition of the spores was not uniform on all tree species, and it was speculated that specific characteristics of tree morphology may have affected the patterns of initial spore deposition and subsequent decrease in viability of the spores of B.t.

Angus et al. (1970, in Forsberg et al. 1976) monitored the numbers of viable spores of B.t. following aerial application of Thuricide 90TS to a mixed balsam fir, spruce, and deciduous forest at 0.6 to 1.4 quarts of formulation (specific activity not given)/acre. They reported that only 15 percent of the spores were viable 24 hours after application and only 5 percent viable 72 hours after application.

Several studies have addressed the potential effects of B.t. on flowers in terms of both phytotoxicity and the effects of the formulations on flower quality. Hamlen and Henley (1976) applied Dipel wettable powder or Thuricide HP, at 1 or 2 lb a.i./100 gal of final spray volume for 4 times at weekly intervals to 10 species of tropical foliage plants. Neither formulation was reported to have any phytotoxic effects. Engelhard (1972) reported no effects on the flower quality of chrysanthemums treated with 2 or 4 quarts of Thuricide 57-428 TC 90 in 100 gal of water, at 66.2 °F (19.0 °C) or at 85.1 °F (29.5 °C), with either 2 or 4 hours drying rate.

3.1.2 Animals

There is little information available on the fate and behavior of B.t. and its toxins in animals. Information regarding the delta-endotoxin is particularly lacking. Studies in several species of animals have shown that B.t. does not persist in the gut of vertebrates following ingestion, but that spores or cells can remain viable in fecal material for as long as 8 weeks (Forsberg et al. 1976). Available studies on the fate and behavior of B.t. formulations and toxins in animals are summarized in appendix H.

Vankova et al. (1974) administered ^{32}P-labeled beta exotoxin of B.t. parenterally (injected into the hemolymph) to caterpillars of the species Galleria mellonella and then monitored the distribution of the measured radioactivity at 20, 60, and 180 minutes after administration. Most of the radioactivity was found in the hemolymph of the caterpillars, with 83.3, 80.6, and 84.7 percent of the radioactivity detected in the hemolymph after 20, 60, and 180 minutes, respectively. Of the remaining ^{32}P label, most was in the skin and in the fat bodies. Parenterally administered material underwent essentially no degradation

Parenterally administered material underwent essentially no degradation in the hemolymph. The ^{32}P-labeled material in the hemolymph was determined by electrophoresis to correspond to the exotoxin. These same authors also reported that ^{32}P-labeled exotoxin, when administered to caterpillars orally by pipette, was degraded in the intestine. In this case, the ^{32}P material was found to pass through the gut wall into the hemolymph and was measured bound to products of ^{32}P-reutilization.

Tojo and Aizawa (1983) reported on the dissolution and degradation of the delta-endotoxin, derived from B.t. var. kurstaki (strain HD-1), as a result of exposure to juice from the gut of the silkworm, Bombyx mori. The original 120,000-dalton subunit of the parasporal crystal was degraded to a toxic protein with a molecular weight of 59,000. In addition, lower molecular weight proteins were produced (less than 40,000) that were not toxic to the silkworm larvae.

Fresh water clams (species not indicated) were found to contain viable B.t. spores when sampled 2 days after 2,000 acres of the rivershed had been treated with Thuricide 16B at a concentration of 4 BIU/acre (9.88 BIU/ha) (Buckner et al. 1974, in Forsberg et al. 1976). No viable B.t. spores were detected in clams from the same location when they were sampled 30 days after application of B.t. formulations, however.

Sebesta and Horska (1973) administered ^{32}P-labeled exotoxin derived from B.t. to mice by the intraperitoneal route. Samples of the urine taken up to 240 minutes after administration of the toxin contained only the exotoxin and small amounts of inorganic phosphate. Excretion of exotoxin in the urine occurred immediately after administration of the labeled material, with a maximum rate of excretion approximately 25 minutes after dosing and with essentially a cessation of excretion 120 minutes after dosing. The authors also reported that the exotoxin levels in various tissues (liver, spleen, heart, intestine, and kidney) decreased rapidly as a consequence of both excretion of the exotoxin and of enzymatic dephosphorylation of the beta-endotoxin to a nontoxic product.

3.2 Soil

Persistence of B.t. in soils was reviewed by Forsberg et al. (1976). B.t. formulations appear to be moderately persistent.

Ignoffo and Graham (1967, in Forsberg et al. 1976) reported a 90 percent reduction in spore count after 4 months for Bakthane L-69 (75×10^9 spores/g) applied to soil exposed to the atmosphere and to

7.28 inches of rainfall. Saleh et al. (1970a and 1970b, in Forsberg et al. 1976) treated various soils with Thuricide T (liquid emulsion of 30 x 10^9 spores/g) and with Biotrol BTB wettable powder (specific activity not provided). They reported recovery of 7,800 to 170,000 spores/g of soil from silty clay and from two silt loams up to 40 days after application. In laboratory soil studies, these authors reported that B.t. spores germinated and exhibited population growth in organically amended soils, but in low pH (5.2) soil that has not been organically amended, the spores germinated while the vegetative cells died.

3.3 Water

There are no reports in the literature regarding the behavior and fate of B.t. formulations and of specific toxins such as the delta-endotoxin in aquatic systems. Buckner et al. (1974, in Forsberg et al. 1976) reported that river water sampled 30 minutes after a 2,500-acre watershed was treated with 4 BIU/acre (9.88 BIU/ha) of Thuricide 16B contained 1,730 spores/ml. Thirty days after application, no B.t. spores were detected in the water. Buckner et al. (1974) reported B.t. concentrations of 22,800 spores/ml after a spray over water. After refrigeration in darkness for 2 months, 7,800 spores/ml were cultured from the same water.

3.4 Air

Few data are available on the behavior and fate of B.t. in the atmosphere. Smirnoff et al. (1973, in Forsberg et al. 1976) reported persistence of B.t. in the air for as long as 17 days after aerial application of B.t. var. thuringiensis (Thuricide HPC) to a mixed coniferous forest. Specific details of amounts and concentrations were not provided.

Results of laboratory studies on guinea pigs (Hazelton Laboratory, reported in Abbott 1986) that breathed Dipel as dust in air for 4 weeks (10.35 mg/l) indicate a slight increase in the incidence and severity in lung lesions as compared to controls. This rate, however, is 20,000 times higher than a bystander could expect during a spray program. Humans exposed to B.t. over 10 years show no adverse effects (Abbott 1986).

4.0 Exposure and Hazard

Forest workers, visitors, and resident or transient animals are all potentially exposed to forestry applications to formulations derived from B.t. For humans and other terrestrial animals, exposures can be by dermal, inhalational, and oral (ingestion) routes. Aquatic animals are potentially exposed by immersion in waterbodies that may contain B.t. In general, it should be noted that environmental exposures resulting from applications of B.t. are of relatively short duration, but any area sprayed in a given year may be sprayed again for several applications in one season or the following year. Occupational exposures, at least for some individuals, may be of longer duration than environmental exposures. However, only a limited number of individuals are so exposed.

Evaluation of the potential for exposure to B.t. by workers and forest visitors in this pesticide background statement is limited by the lack of available data from specific studies on exposure to B.t. In addition, it is difficult to extrapolate data from chemical pesticides to B.t. formulations, which are biological insecticides. Any valid understanding of the potential hazard from use of formulations based on B.t. must consider specific toxicological effects of the various stages of the life cycle to which exposure may reasonably be assumed to occur. This section summarizes available information on the potential for exposure to B.t. and, based on the available information, assesses the potential hazard from its use in terms of both occupational and environmental exposure.

It should be noted that B.t. is a naturally occurring pathogenic organism that is normally found in the environment, although it is found at relatively low ambient levels compared to levels that occur after application of pesticidal formulations derived from B.t. In general, the limited data that are available indicate that the spores and crystals of B.t., which constitute the active ingredients of pesticidal formulations used by the USDA Forest Service, appear to be resistant to environmental degradation. B.t. formulations are moderately persistent in most soil environments but are readily diluted. B.t. has been reported in air for as long as 17 days after aerial application, but actual concentrations were not indicated. There are no reports on the behavior of B.t. in aquatic systems. B.t. is rapidly passed through the gut of vertebrates with little, if any, absorption.

4.1 USDA Forest Service Application of *Bacillus thuringiensis*

Available application data for use by the USDA Forest Service of formulations derived from B.t. are presented in table 4-1. Actual

Table 4-1

USDA Forest Service Application Data for *Bacillus thuringiensis* for 1983

Method of Application (Number of Projects)	Average Number People Exposed Per Project	Total Number of People Exposed Per Application	Duration of Exposure Per Application				Type of Management (Projects)
			Average		Maximum		
			Hrs/Day	Days/Yr	Hrs/Day	Days/Yr	
Aerial (2)	100	199	2.25	7.5	4.5	8.5	Timber (2)

Source: Gross 1984.

exposure to such formulations differs among Forest Service personnel involved in their use, other Forest Service personnel, visitors to Forest Service lands that have been treated with *B.t.*, and wildlife species that are permanent or transient inhabitants of treated areas.

The data presented in table 4-1 indicate the number of forest workers involved in the application of *B.t.* formulations in 1983, as well as the duration of exposure on a daily and an annual basis. However, the actual exposure of these workers depends not only upon the specific formulation, method, and rate of application, but also on the protective measures employed, the operating condition of the equipment used during application, and accidental events which expose workers to the pesticide.

4.2 Exposure

The following discussion of exposure to *B.t.* and its formulations considers both occupational exposure and environmental exposure. The former is the potential for exposure by forestry workers during application of *B.t.* formulations. The latter is the potential for exposure to the general public and to wildlife species.

Table 4-2 summarizes estimates of potential exposure to *B.t.* and its formulations as a result of aerial application. For purposes of calculation, it is assumed that an application rate of 8.4 BIU/acre, equivalent to 1.2 lb/acre, is utilized. Although the application rate used by the USDA Forest Service is 14 to 16 BIU/acre (table 1-2), the higher rate is recommended for forest use for control of gypsy moths.

4.2.1 Occupational Exposure

Data are not available on the daily occupational exposures or doses of *B.t.* formulations to forestry workers. *B.t.* formulations used by the USDA Forest Service are based on parasporal crystals of *B.t.* var. *kurstaki*. These formulations do not contain the beta-exotoxin or the alpha exotoxin, both of which are toxic to vertebrates, including humans. Available studies indicate that the delta-endotoxin is not toxic to vertebrates.

4.2.2 Environmental Exposures

Environmental exposures of humans to *B.t.* can occur as a result of direct contact with sprayed foliage, inhaling spray mist, eating plants or animals contaminated with *B.t.* formulations, or drinking water containing *B.t.* cells. Animals, both terrestrial and aquatic, also are subject to the same environmental exposures as well.

Table 4-2

Summary of Occupational Dose, Environmental Exposure and Toxicity for *Bacillus thuringiensis*

	Exposure Route (mg/kg/day)[a]	
	Dermal/Inhalation	Oral
Occupational Dose		
Mixer/Loader	ND	ND
Observer	ND	ND
Backpack Sprayer	ND	ND
Environmental Exposure		
Fish (immersion)	0.012	0.012
Rabbits	0.484/negligible	1.26
Deer	0.121/negligible	1.73
Humans (ingestion)[b]		
Fish	NA	0.00002
Rabbits	NA	0.005
Deer	NA	0.007
Water	NA	0.0005
Berries	NA	0.001
Mushrooms	NA	0.0006
Toxicity Summary	No dermal/inhalation toxicity reported.	No acute, chronic or toxicity data for only delta-endotoxin.

[a] Assumes aerial application at 8.4 BIU/acre (about half the normal application rate).

[b] Human environmental exposures via dermal and inhalation routes will be considerably less than occupational doses or exposures. Oral environmental exposures are estimated for the six sources listed.

ND - no data available, NA - nonapplicable data.

For purposes of estimating potential environmental exposures for humans and animals as a result of forestry application of formulations derived from B.t., the following analysis makes certain assumptions that are necessary due to the unique nature of B.t. formulations. For example, it is assumed are that an application rate equivalent to 8.4 BIU/acre is used. This rate is considerably lower than the rate used by the USDA Forest Service (14 to 16 BIU as indicated in table 1-2) but was the rate recommended for forestry application of suspension formulations for the control of western spruce budworm (Forsberg et al. 1976).* It is also assumed that the applied B.t. formulation contains 30 billion viable spores/g and 7.2 BIU's/lb (Dipel WP, table 1-1). It is important to note, however, that as a biological agent, there is a considerable variation in the potency and toxic activity of different formulations as a result of differing strains of B.t. from which they are derived.

4.2.2.1 Dermal Exposures. Casual forest visitors' dermal environmental exposures would be less than occupational exposures, since spray operators and observers are directly involved with actual application activities on a regular basis on the spray units. A casual visitor to spray units should be expected to receive direct exposure that is orders of magnitude less than that received by an observer receiving direct spray deposition throughout the spray season.

Animals in the target spray zone are subject to dermal exposure as a result of aerial application of B.t. and from direct contact with foliage that has been treated with B.t. Rabbits and deer, representing both small and large game animals, respectively, have the following dermal exposures based on an aerial spray application rate of 8.4 BIU (1.2 lb)/acre.

Rabbits in Aerial Spray Zone - 0.484 mg/kg (0.403 mg/kg at 1 lb/acre application rate)

Deer in Aerial Spray Zone - 0.121 mg/kg (0.101 mg/kg at 1 lb/acre application rate)

The following assumptions were made:

- 1.0-lb/acre application rate, based on recommended rate of 8-16 oz/acre for Thuricide 64 LV.
- deer equivalent in size to 88-lb (40 kg) goat and 2.3 m^2 surface area.

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*Recommended rate in 1986 is 12 BIU/acre (30 BIU/ha) (Sandoz 1986).

- rabbit weight 2 kg and .23 m^2 surface area.
- $mg/m^2 \times 0.23\ m^2/rabbit \times rabbit/2\ kg = y\ mg/2\ kg$ (from U.S. Forest Service 1985)

4.2.2.2 Inhalation Exposures. Human inhalation environmental exposures would be less than occupational exposures, since spray operators, involved with activities on the spray units, are more likely to be subject to spray mist than is a casual visitor. Environmental inhalation exposures of humans and animals in the spray target area would occur on a one-time basis and would be short because of the rapid dilution. Inhalation exposures are expected to be small and they are neglected in this analysis.

4.2.2.3 Immersion Exposures. Aquatic organisms are subject to environmental exposures from immersion in streams and ponds adjacent to target spray zones. Environmental exposures for aquatic organisms would be equivalent to the estimated concentration of *B.t.* formulations in water. This estimated concentration is derived as follows, assuming water concentration at 0.012 ppm (0.01 ppm at 1 lb/acre application rate). This concentration of *B.t.* represents the typical concentration immediately after aerial application. Buckner et al. (1974) reported water concentrations following a *B.t.* spray were 22,800 spores/ml. Therefore, assume 30 billion spores/acre foot of water. If heavy rainfall were to occur immediately after such application, higher transient concentrations could occur in streams and ponds, but such concentrations would be rapidly lowered by dilution, transport, and possibly by biological degradation.

4.2.2.4 Oral Exposures. In terms of oral exposures, any incidental ingestion of *B.t.* by workers on the spray unit would be minimal, and ingested material would be rapidly eliminated in feces. Oral environmental exposures would occur for wildlife eating contaminated vegetation and for humans consuming of fish, deer, rabbit, water, berries, and mushrooms. Similarly, any material ingested by fish and wildlife would be rapidly eliminated in the feces. Basic assumptions for estimates of oral environmental exposures for both man and wildlife are presented in the General Introduction.

Oral exposures for deer and rabbits, representing game animals potentially eaten by humans, assume vegetation is sprayed aerially at a rate equivalent to 8.4 BIU (1.2 lb)/acre and that rabbit browse is partially shielded by overstory. These estimates are as follows:

Deer = 1.73 mg/kg (1.44 mg/kg at 1 lb/acre application)

Rabbit = 1.26 mg/kg (1.05 mg/kg at 1 lb/acre application)

Estimates of human oral exposures from consumption of fish, deer, and rabbit meat require, as a starting point, estimates of maximum tissue concentrations of B.t. in fish, deer, and rabbit.

Fish tissue concentrations are derived as follows:

- water concentrations = 0.012 ppm.
- bioconcentration = 0.1 because material is passed out from the gut in most vertebrate animals studied.

Fish tissue concentration = 0.0012 ppm (0.0012 mg/kg).

Deer and rabbit tissue concentrations include B.t. accumulated from both oral and dermal exposure. Tissue concentration estimates for deer and rabbit assume that 1 percent of dermally applied B.t. is absorbed, since these animals are protected by fur. It is a also assumed that oral exposure of deer and rabbit to B.t. lasts for only 2 days since the available data indicate a moderate persistence for B.t. formulations in most environmental media. It is further assumed that only 10 percent of the ingested B.t. is assimilated by the animal since available data indicate that spores and crystals of B.t. are passed through the vertebrate gut via the feces. Deer tissue concentrations of B.t. are derived as follows:

Dermal Exposure contribution assumes 1 percent absorption of a single exposure, or 0.121 mg/kg (dermal exposure) x 0.01 = 0.001 mg/kg.

Oral Exposure contribution assumes 10 percent assimilation of 2-days feeding, or 1.73 mg/kg/day (oral exposure) x 0.1 x 2 days = 0.346 mg/kg.

Total Deer Tissue concentration = 0.0012 mg/kg + 0.346 mg/kg = 0.347 mg/kg (0.347 ppm).

Rabbit tissue concentrations of B.t. are derived in the same way as for deer, as follows:

Dermal Exposure contribution assumes 1 percent absorption of a single exposure, or 0.484 mg/kg (dermal exposure) x 0.01 = 0.005 mg/kg.

Oral Exposure contribution assumes 10 percent assimilation of 2-days feeding, or 1.26 mg/kg/day (oral exposure) x 0.1 x 2 days = 0.252 mg/kg.

Total Rabbit Tissue concentration = 0.005 mg/kg + 0.252 mg/kg = 0.257 mg/kg (0.257 ppm).

Based upon the above fish, deer, and rabbit tissue concentrations and human consumption rates, the following estimates of maximum human oral exposures to B.t. from eating meat are derived:

Fish Meat - 0.002 mg/kg x 2 days x 0.5 kg/day/person divided by 50 kg person - 0.00002 mg/kg.

Deer Meat - 0.347 mg/kg x 2 days x 0.5 kg/day/person divided by 50 kg person - 0.007 mg/kg.

Rabbit Meat - 0.257 mg/kg x 2 days x 0.5 kg/day/person divided by 50 kg person - 0.005 mg/kg.

Water and plant consumption by humans would result in the following oral exposures to formulations derived from B.t., based on assumptions stated in the General Introduction at an aerial application rate of 8.4 BIU (1.2 lb/acre).

Water - 0.00048 mg/kg (0.0004 mg/kg at 1 lb/acre application).

Berries - 0.0012 mg/kg (0.001 mg/kg at 1 lb/acre application).

Mushrooms - 0.0006 mg/kg (0.0005 mg/kg at 1 lb/acre application).

4.3 Hazard Assessment

Available data on the toxicological properties of B.t., of its major exotoxins and endotoxins, and of pesticidal formulations derived from B.t. are summarized in table 2-1 and detailed in section 2 of this insecticide background statement. Table 4-2 provides estimates of environmental exposures in humans and wildlife. There is no available toxicity information for B.t. formulations based on B.t. var. kurstaki, as used by the USDA Forest Service, that are appropriate for comparison to these estimates.

The available studies in the scientific literature indicate that B.t. formulations used by the USDA Forest Service have a low toxicity to vertebrates and nontarget organisms and are nonmutagenic. The alpha-exotoxin, which is toxic to vertebrates, is not produced by B.t. var. kurstaki, the strain from which these formulations are derived. The beta-endotoxin, which is also toxic to vertebrates, is denatured during the production of pesticidal formulations. There are no studies available in the literature indicating carcinogenicity, reproductive toxicity, or teratogenicity for B.t. or its formulations.

Despite this lack of evidence for toxic effects from B.t. formulations, care should be exercised during its use. Samples and

Buettner 1983 and Warren et al. 1984 (both in USDA Forest Service 1984) reported isolation of vegetative cells and spores of B.t. following accidental occupational exposures to B.t. Cells and spores were isolated from a skin infection that resulted when a laboratory technician was accidentally stuck with a needle contaminated with B.t. var. israelensis. Cells and spores were also isolated from an eye ulcer following an accidental splashing of B.t. into the eye of a farm worker. Any possible cause for concern is the potential for vegetative cells of B.t. to produce the beta-exotoxin in sufficient quantities to be toxic to vertebrates and humans. However, there is no evidence to indicate that natural populations of B.t. produce these toxins in sufficient quantity to pose a potential hazard to nontarget organisms.

Appendix A

Summary of Bacillus thuringiensis Toxicity Data for Microorganisms

Appendix A

Summary of Bacillus thuringiensis Toxicity Data for Microorganisms

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Dipel	Mouse fibroblast cells and vesicular stomatitis virus	Dipel and vehicle tested in dilutions from <185 to 100,000 ppm	18 hrs prior to challenge	No significant enhancement recorded, product was noncytotoxic in ppm <185, concs. of 550 to 100,000 ppm proved to be cytotoxic.	Details of study unavailable.	Appl. Environ. Microbiol. 35:297, 1978
B.t. var. thuringiensis (Thuricide 90T)	Soil microorganism	Soil, alfalfa and B.t. mixed, put in petri plate, moistened, 25 °C; pH varied	Incubated 4 days	B.t. can germinate and compete vegetatively with soil microorganisms and sporulate successfully to 1×10^6 spore/g soil and higher.	No survival if conditions unfavorable to competitive soil bacilli.	Saleh et al. 1969

Appendix B

Summary of Bacillus thuringiensis Toxicity Data for Invertebrates

Appendix B

Summary of *Bacillus thuringiensis* Toxicity Data for Invertebrates

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
B.t. var *thuringiensis* (delta-endotoxin)	Honey bee, *Apis mellifera*	Oral, *ad lib*. in sucrose	Not given	No mortality, paralysis, or cessation of feeding among bees fed 0.5 to 16.0 x 10^6 (units not given, assumed to be spores from context)/bee.	Brood and adults tested.	Cantwell et al. 1966 in Forsberg et al. 1976
B.t. var. *alesti* (delta-endotoxin)	Honey bee, *Apis mellifera*	Oral, *ad lib*. in sucrose	Not given	No detectable mortality or ill effects among bees fed 0.5 x 10^6 (units not given, assumed to spores from context)/bee.	Brood and adults tested.	Cantwell et al. 1966 in Forsberg et al. 1966
B.t. var. *sotto* (delta-endotoxin)	Honey bee, *Apis mellifera*	Oral, *ad lib*. in sucrose	Not given	No detectable mortality or ill effects among bees fed 0.25 to 0.50 x 10^6 (units not given, assumed to be spores from context)/bee.	Brook and adults tested.	Cantwell et al. 1966 in Forsberg et al. 1966

Appendix B (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Dipel (wettable powder, specific activity not given, variety of B.t. or serotype not indicated but presumed to be HD-1)	Parasitic wasps (Braconidae, Chalcididae, and Ichneumonidae) and flies (Tachinidae and Sarcophagidae)	Two applications of 20 BIU/ha by aerial spray.	Insects sampled in year of application and 1 yr later.	Significantly fewer adults of 2 of 3 species of Tachinidae, Branonidae, Chalcididae, and Sarcophagae ($p < 0.05$) were reported in treated areas compared to untreated controls.	176 ha treated in NW New Jersey.	Reardon et al. 1979
Dipel (B.t. var. alesti, HD-1 5% wp), specific activity not given)	Predatory insect, spined stiltbug (Jalysus spinosus)	B.t. applied twice at approximately 5-wk intervals to tobacco.	Sampled up to 4 days after last application.	B.t. applied at a rate of 5 lb/acre did not reduce the numbers of stiltbugs.	NA	Elsey 1973
Thuricide (specific activity not given, variety of B.t. or serotype not indicated but presumed to be HD-1)	Various insect parasitoids (Hymenoptera (wasps); Diptera (flies); Neuroptera (lacewings); and Coleoptera (beetles)).	Insects exposed to dried formulation on filter paper discs. B.t. applied to paper at a rate equivalent to 40 BIU/ha.	Up to 120 hrs	No mortality reported for adults of the wasp genera Campoletis, Metecrus, Brachymeria, or Chelonus, or the fly genus Voria. Adult beetles (genus Hippodamia) showed 3.3% mortality. In the lacewing (Chrysopa), there was 3.3% mortality among adults and no mortality among larvae.	NA	Wilkinson et al. 1975

Appendix B (Concluded)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Thuricide (4,000 1 μ/mg serotype not given but presumed to be HD-1)	Parasitic wasp; *Trichogramma nubilale*	Bioassay with treated pepper plants. Residues weathered in greenhouse for up 21 days.	3 days	*B.t.* applied to leaves at a rate of 9.8 BIU/ha did not result in reduced emergence from, or parasitism of, European corn borer eggs.	NA	Tipping and Burbutis 1983
B.t. var. *kurstaki*; 18,000 1 μ/mg	Chinese praying mantis, *Tenodera aridifolia sinensis*	Mantids fed diet of cabbage looper larvae fed for 15 hrs on a diet of 150 μg/ml of *B.t.* formulation.	Up to 5 days, then observed for 7 days.	No mortality or abnormal behavior among mantids fed looper larvae that had consumed a lethal dose of *B.t.*	A sample of treated looper larvae, returned to normal diet. A total of 29 to 32 died.	Yousten 1973
Dipel (16,000 1 μ/mg, serotype "H3") (Note: see comments)	Earthworms (no genera specified)	Application to forest soil.	Up to 9 wks	Dipel applied at rates of 60, 600, or 6,000 mg/m^2 did did not adversely affect populations of earthworms.	Study also included effects Bactospeine (serotype H1; 1,000 1 μ/mg). At 30 g/m^2, it did not adversely affect worm populations.	Benz and Altwegg 1975

NA - data not available.

Appendix C

Summary of Bacillus thuringiensis Toxicity Data for Fish

Appendix C

Summary of Bacillus thuringiensis Toxicity Data for Fish

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Thuricide 56-54B (SS-low potency 16B-type form.) (1.2 BIU/qt)	Rainbow trout Black bullhead Yellow perch	Acute LC_{50}	96 hrs	77 ppm 1700 ppm 920 96H LC_{50}	--	Sandoz[a] (unpublished)
Thuricide 56-549 (90TS) (2.0 BIU/qt)	Rainbow trout Back bullhead Yellow perch			370 ppm 800 ppm 480 pm 96H LC_{50}	--	Sandoz (unpublished)
Thuricide No. 57-18 (sample #58181 from production batch 1F x 8)	Mosquito fish	Acute toxicity 1 to 100 ppm	120 hrs	No effect	--	Sandoz (unpublihed)
Thuricide Tech. Conc. 65B	Rainbow trout	Acute toxicity 1 to 10 ml/kg water		No effect	--	Sandoz (unpublished)
Thuricide	Rainbow trout	Acute toxicity	14 days	No effect	--	Sandoz (unpublished)
Thuricide Tech. Conc.	Coho salmon juveniles	Median tolerance 8 to 406 mg/l water	168 hrs	48 hour median tolerance limits: Thuricide - 50 mg/l DDT - 0.08 mg/l.	Thuricide 1/30 as toxic as DDT to fish.	Sandoz (unpublished)
Thuricide Wettable Powder	Periwinkle, mussels, oyster, Artemia salina, shrimp, Pomatoschistus minutus	Tests with various marine species 0, 10, 50, 100, 200 and 400 mg/l	96 hrs	Except for a slight increase in mortality against Artemia salina at the highest rate tested, the results indicated that Thuricide does not present any hazard of acute toxicity for any of the marine species tested.	--	Sandoz (unpublished)

Appendix C (Concluded)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Thuricide; Thuricide + chitinase; chitinase alone and Dipel with and without chitinase	Brook trout *Salvelinus fontinalis*; White sucker *Catostomus commersoni*; small mouth bass *Micropterus dolomieui*	Aerial treatment 2,500 acres (rate of application unknown).	--	Monitoring at 3 days and 1 mo following observed normal fish populations and behavior; no adverse effects.	Temporary knockdown of fish foods (midge and mayfly) due to physical interaction with spray product. product.	Buchner et al. 1974
B.t. var. *israelensis*	Rainbow trout *Salmo gairdnerii*	6 bioassay vessels 10 trout/12.5 l water; aerated at 48 hrs; treatment 3 groups 370 mg/l, 3 groups 300 mg/l, 1 control.	96 hrs	1 mortality between 72 to 96 hrs at 370 mg/l; no observed difference between tests and control; no LC_{50} if 300 mg/l produces less than 50% mortality.	Details of study unavailable.	Toxigenics 1981a
B.t. var. *israelensis*	Bluegill *Lepomis macrochirus*	12 bioassay vessels with 5 bluegill/12.5 l water; aerated; treatment; 6 groups 600 ppm, 6 groups 300 ppm, 2 controls.	96 hrs	1 mortality each in 2 vessels at 72 hrs, 1 mortality each in 3 vessels at 96 hrs for 300 ppm. 2 mortalities at 72 hrs and 5 mortalities at 96 hrs for 600 ppm; no LC_{50} if 300 mg/l (ppm) produces 50% mortality.	Details of study unavailable.	Toxigenics 1981b

[a] Report not available; only petition summary.

Note: Due to the time frame in which some of these studies were conducted (pre-EPA *B.t.* Tolerance Exemption), Thuricide formulations may have contained beta-exotoxin.

Source: Sandoz Crop Protection Corp. unpublished.

Appendix D

Summary of Bacillus thuringiensis Toxicity Data for Birds

Appendix D

Summary of Bacillus thuringiensis Toxicity Data for Birds

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Thuricide (50 x 10^9 B.t. spores/g, no further data provided. Assumed to be only delta-endotoxin from context)	Mallards	Acute oral	--	LD_{50} >>2,000 mg/kg	--	Tucker and Crabtree 1970
Dipel WP (B.t. var. kurstaki specific activity not given)	Chickens (2-wk-old pullets)	Oral in diet	Ingestion of treated rations for 2 wks followed by normal rations.	Birds fed 0 (control); 0.5, 1.0, 2.0, or 4.0% B.t. formulation showed weight gains of 351, 319, 218, 114, and 50 g, respectively, after 14 days.	ome effects may be due to food avoidance at concs. of 2% or 4% B.t. but no supporting data given.	Couch 1973, in Forsberg et al. 1976

Appendix D (Concluded)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
B.t. var. *israelensis*	Mallard	Drinking water 1.48 g/l twice daily, rate of exposure was 2000X application rate.	14 days	Behavior normal; no gross pathological changes; no absorption from GI tract. Tissues negative for microbial (liver, kidney, spleen, and lung).	No details provided, no data.	Abbott Laboratories Exp. 912-1986 summary[a]
Dipel	Bobwhite quail	None given, dose >10 g *B.t.*/kg body weight.	--	No pathology attributed to *B.t.*	No details provided.	Abbott Laboratories Toxicology profile 1985[a]
Thuricide 16B and Dipel WP	15 families of songbirds	Aerial spray 2 to 4 gal/ac *B.t.* with and without chitinase and chitinase alone plots.	1 mo	Several species of warblers and sparrows varied (not significant) from norm in treatment plots; no significant reduction in bird populations.	High rate of application.	Buckner et al. 1974

[a] Research results not available, only summary provided.

Appendix E

Summary of Bacillus thuringiensis Toxicity for Mammals

Appendix E

Summary of *Bacillus thuringiensis* Toxicity Data for Mammals

Formulation	Organism	Nature of Exposure	Effects	Comments	Reference
			Acute Oral Toxicity		
B.t.	Mouse	Administered orally in 20% suspension in water to three groups of 10 females at 0, 0.3, and 15 x 10^6 IU/kg. The 15 x 10^6 IU contained 27 to 120 x 10^{12} spores. Animals observed for 7 days.	No deaths were observed.	Test material may have included the beta-exotoxin.	Kimura 1970 in Forsberg et al. 1976
Thuringiensin (beta-exotoxin)	Mouse	Oral administration of 100, 200, 400, 800, 1,600, or 3,200 μg of material to groups of 20 female mice (14 to 16 g body wt).	An LD_{50} was not reached even when up to 200 μg/g body wt was fed consecutively for 8 days.	No mortalities were observed at 100 μg/g and animals were reported to tolerate at least 800 μg.	de Barjac and Riou 1969 and in Forsberg et al. 1976 and in Sebesta et al. 1981
Thuricide	Mouse	Two groups of 5 mice fed rations containing 15% Thuricide (93 x 10^9 spores/g) for 7 days, then sacrificed. Results were compared to two control groups.	No gross pathological lesions were reported. *B.t.* cells were isolated from tissues (heart, liver, spleen, kidney, and lung) of treated animals but not from controls.	Test material contained beta-exotoxin as a toxic agent.	Stewart 1961 in Forsberg et al. 1976
Thuricide	Hog	Four animals fed treated feed for five consecutive days. Thuricide added to feed at a level of 7.5 x 10^{11} spores/day.	No pathogenicity or effect on wt gains were reported.	Test material contained beta-exotoxin as a toxic agent.	Briggs 1959 in Forsberg et al. 1976

Appendix E (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	Reference
			Acute Oral Toxicity (Continued)		
Thuringiensin (beta-exotoxin)	Rat	A 33% suspension containing 1% carboxymethylcellulose as a thickener administered by gavage. Doses up to 24 g (estimated 2 x 10^{12} viable spores)/kg body wt were administered to groups of 10 rats. Animals were observed for 1 wk.	No fatalities or any outward symptoms of toxicity were observed. No differences between the tissues of test and control animals were observed following gross and histological examination.	Test material contained beta-exotoxin as a toxic agent.	Fisher and Rosner 1959
Thuricide	Rat	Single doses of 6, 12, and 24 g/body wt administered orally to 2 and 10 10 animals, respectively.	No pathogenicity was reported. No gross or histopathological lesions were observed in lungs or other organs from one test rat given the highest dose or from one control rat.	Test material contained beta-exotoxin as a toxic agent.	Hixson 1957 in Forsberg et al. 1976
Biotrol	Human	Five male and 5 female volunteers fed 1 g Biotrol (10 x 10^9 spores) in diet for 3 consecutive days.	Physical, hematological, and clinical tests were normal.	Test material may have contained beta-exotoxin as a toxic agent. B.t. was detected in feces of 5 individuals.	Long et al. updated in Forsberg et al. 1976
Thuricide	Human	Thirteen volunteers ingested 1 g Thuricide (3 x 10^9 spores/g) by capsule daily for 5 days.	All physical examinations were normal (including blood pressure; respiratory and pulse rates; evaluations of genitourinary, gastrointestinal, cardiopulmonary, and nervous systems; and blood and urine laboratory analysis.)	Test material contained beta-exotoxin as a toxic agent.	Fisher and Rosner 1959

Appendix E (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	Reference
			Acute Oral Toxicity (Concluded)		
Biotrol	Mouse	Six groups of 6 mice were fed Biotrol (10^{10} spores/g added to feed at levels of 0, 0.5, 1.0, 2.0, 4.0, and 8%. Animals were observed for 11 days).	No toxic effects were reported.	Test material may have contained beta-exotoxin as a toxic agent.	Williams and Hernandez undated in Forsberg et al. 1976
Biotrol	Rat	Four groups of 2 males and 2 females were fed Biotrol added to 5 g of stock diet at a rate of 0, 2.5, 5, and 10 g/kg body wt for 1 days feed. Animals were observed for 13 days.	No deaths or effects on weight gain were reported. Blood counts were normal.	Test material may have contained beta-exotoxin as a toxic agent.	Hernandez and McLean undated in Forsberg et al. 1976
Biotrol	Cattle	Steers orally administered 2.7 x 10^{13} spores/day.	No effects were reported.	Test material may have contained beta-exotoxin as a toxic agent.	Dunn 1960 in Forsberg et al. 1976
Dipel	Dog	Three groups of 3 female dogs each were orally administered 0, 1.2 x 10^5, and 6 x 10^6 IU/kg body wt.	No deaths were reported after 48 hrs observation.	The highest dose was equivalent to 400 mg/kg body wt. 1.5 x 10^4 IU Dipel contained between 27 x 10^6 and 120 x 10^6 viable spores.	Kimura 1970 in Forsberg et al. 1976

Appendix E (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	Reference
			Acute Parenteral Toxicity		
B.t. washed vegetative cells or spores of four strains	Mouse	Vegetative cells and spores were injected intraperitoneally (i.p.) or subcutaneously (s.c.).	LD$_{50}$'s for B.t. thuringiensis cells 8.4 x 10^6 and 9.0 x 10^7 by i.p. and s.c. routes, respectively and for spores were 3.7 x 10^8 and 1.4 x 10^9 by i.p. and s.c. routes, respectively. LD$_{50}$'s for B.t. sotto cells were 1.1 X 10^7 and 1.1 x 10^8 by i.p. and s.c. routes, respectively. B.t. sotto spores were nonlethal by both routes "... at the concs. tested." LD$_{50}$'s for B.t. alesti cells were 4.7 x 10^6 and 9.0 x 10^7 by i.p. and s.c. routes, respectively and for spores were 1.5 x 10^8 and 8.0 x 10^9 by i.p. and s.c. routes, respectively. LD$_{50}$'s for B.t. entomocidus cells were 6.3 x 10^5 and 1.9 x 10^6 by i.p. and s.c. routes, respectively and for spores were 1.6 x 10^8 and 1.0 x 10^9 by i.p. and s.c. routes, respectively.	NA	Lamanna and Jones 1963 in Forsberg et al. 1976

Appendix E (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	Reference
			Acute Parenteral Toxicity (Continued)		
Suspension of B.t. derived from Thuricide	Mouse	I.P. injection of 3×10^8 viable organisms in 1 ml of culture into each of 5 mice.	No deaths or abnormal symptoms were observed.	Test material may have contained beta-exotoxin. In addition, the material was washed with isotonic saline and was free of carrier material.	Fisher and Rosner 1959
Autoclaved suspension of B.t. derived from Thuricide	Mouse	I.P. injection of 3×10^8 nonviable organisms into each of 5 mice.	All mice died within 16 hrs following of abdominal irritation and sensitivity.	Test material was autoclaved prior to injection. The authors attributed death to the carrier only.	Fisher and Rosner 1959
Suspension of B.t. derived from Thuricide	Mouse	Five mice were injected i.p. with 1 ml of broth culture. After 6 hrs, 0.3 ml of blood was withdrawn from each mouse and injected i.p. into second group of 5 mice. Procedures repeated for six transfers.	No wt loss or toxic symptoms were observed. One mouse in the initial group died within 4 hrs of injection and showed severe irritation of peritoneum on autopsy. The remaining mice in this initial group exhibited malaise.	Organisms survived the passages but showed no evidence of increased virulence.	Fisher and Rosner 1959
B.t. culture broth containing alpha-exotoxin	Mouse	I.P. injection of 2 ml of a broth culture containing alpha-exotoxin. Number of animals not specified.	100% mortality within 2 hrs.	Initial symptoms of shock (excited state followed by labored breathing) preceded death.	Kreig 1970 in Forsberg et al. 1976

Appendix E (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	Reference
			Acute Parenteral Toxicity (Continued)		
Purified beta-exotoxin from B.t. variety gelechiae	Mouse	Group of 15 mice injected i.p. with 0.2 ml of purified exotoxin. Specific dose not stated.	LD_{50} was 18 μg/g body wt. Mice died on 3rd day post injection.	No histological pathology was seen in brain, liver, lymphatic system, or bone marrow.	Sebesta et al. 1969 in Forsberg et al. 1976
B.t. thuringiensis	Mouse	Groups of 20 mice injected i.p. with 0.5 ml beta-exotoxin at doses of 100 to 800 μg.	LD_{50} was 13.3 μg/g body wt.	Hepatic necrosis was observed at autopsy.	de Barjac and Riou 1969 in Forsberg et al. 1976
B.t. thuringiensis	Mouse	Groups of 20 mice injected subcutaneously (s.c.) with 0.5 ml beta-exotoxin at doses of 100 to 800μg.	LD_{50} was 16.6 μg/g body wt.	Hepatic necrosis was observed at autopsy.	de Barjac and Riou 1969 in Forsberg et al. 1976
Thuricide	Guinea Pig	Ten animals injected i.p. with 4 ml of a 24-hr-old culture. Animals were observed for 7 days.	No deaths.	Test material may have contained beta-exotoxin as a toxic agent.	Fisher and Rosner 1959
Thuricide	Guinea Pig	Ten animals injected i.p. with 1 ml of washings from glucose agar slants containing a relatively high conc. of bacteria. (Numbers unknown). Animals were observed for 7 days.	Seven animals died during the observation period.	Test material may have contained beta-exotoxin as a toxic agent.	Fisher and Rosner 1959

Appendix E (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	Reference
			Acute Parenteral Toxicity (Concluded)		
Biotrol	Guinea Pig	Twenty animals each injected i.p. with a single dose of 5 x 10^8 spores.	No deaths after 14 days. B.t. was detected in heart blood, peritoneum, spleen, liver, and kidneys at autopsy.	Test material may have contained beta-exotoxin as a toxic agent.	Holland 1959 in Forsberg et al. 1976
Biotrol	Rabbit	Eight rabbits given single i.p. injection of 1 x 10^9 spores.	No deaths after 14 days. B.t. was detected in heart blood at autopsy.	Test material may have contained beta-exotoxin as a toxic agent.	Holland 1959 in Forsberg et al. 1976
Biotrol	Hog	Four animals injected i.p. with a single dose of 2 x 10^9 spore. Animals sacrificed after 4 days.	No deaths after 4 days. B.t. was detected in heart blood and peritoneum at autopsy.	Test material may have contained beta-exotoxin as a toxic agent.	Holland 1959 in Forsberg et al. 1976
			Dermal Irritation		
Dipel	Rabbit	20% suspension applied on shaved shaved and abraded skin.	No irritation after 3 days.	--	Kimura 1970 in Forsberg et al. 1976
			Inhalation		
Thuricide	Mouse	Ten animals were subjected to 4 inhalation exposures of 15 min each over a period of 6 days. Ten grams of material were dispersed with a powder blower during each exposure in a 30 x 30 x 30 cm chamber.	No toxic effects were observed. Gross pathology findings were negative. Normal wt gains were observed.	Test material contained beta-exotoxin as a toxic agent. Ten animals were also exposed to autoclaved material to determine if the carrier had any effect on lung irritation. This test was also negative.	Fisher and Rosner 1959

Appendix E (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	Reference
Inhalation (Concluded)					
Dipel	Mouse, Rat, Guinea Pig	5-10 animals/group were exposed to aerosol of a 20% suspension for 10 min.	No mortalities were observed among test animals after 1 wk.	--	Kimura 1970 in Forsberg et al. 1976
Inhalation and Ingestion					
Thuricide	Human	Five individuals ingested 1 g of Thuricide containing approximately 3 x 10^9 spores, daily for 5 days and also inhaled 100 mg of test material daily for 5 days.	No adverse effects were observed. All laboratory findings were negative. Examinations included records of wt; blood pressure; respiratory rate vital capacity (inhalation group); evaluation of genitourinary, gastrointestinal, cardiorespiratory and nervous systems. Laboratory tests included urinalysis, quantitative urobilinogen determination, complete blood count, sedimentation rate, blood urea nitrogen, glucose, bilirubin, and thymol turbidity tests.	Test material contained beta-exotoxin as a toxic agent.	Fisher and Rosner 1959

Appendix E (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	Reference
		Acute Eye Irritation			
Dipel	Rabbit	0.1 ml of a 2% suspension by ocular instillation.	No irritation was observed.	--	Kimura 1970 in Forsberg et al. 1976
Dipel	Human	An 18-yr-old farmer accidentally splashed a suspension of the test material in his right eye. The eye was immediately irrigated with water and treated with antibiotic ointment.	Eye became irritated and was treated with corticosteroid ointment 3 days after exposure. The cornea became ulcerated 10 days after the accident. *Bacillus thuringiensis* was isolated from the lesion, which healed after 2 wks of topical treatment with Gentamicin.	NA	Samples and Buettner 1983
		Subchronic Toxicity			
Spent culture medium from *B.t.*	Mouse	Oral administration for 3 mos.	No pathological changes were observed.	NA	Carlberg 1973 in Sebesta et al. 1981
Dipel	Rat	One group of 10 animals fed 0.84 mg Dipel/kg diet/day for 1 wk, then 840 mg/kg diet/day. A 2nd group was fed 8.4 mg/kg diet/day for 1 wk, then 8,400 mg/kg/day. A 3rd group served as controls. Detailed hematology, blood chemistry and urine analysis performed at 0, 4, and 13 wks. Gross and histopathology conducted at autopsy.	No significant differences observed between test and control animals for any of the parameters measured.	--	Olson and Kwapien 1973 in Forsberg et al. 1976

Appendix E (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	Reference
Subchronic Toxicity (Concluded)					
Biotrol	Rat	Groups of 20 (10 of each sex/group) were each fed Biotrol in diet at levels of 0, 1.25, 2.5, 5, and 10% for 49 days.	No differences between test and control animals.	Test material may have contained beta-exotoxin as a toxic agent.	Hernandez and Muckenthaler undated in Forsberg et al. 1976
Dermal Sensitization					
Suspension of Thuricide	Guinea Pig	0.1% suspension in water injected intracutaneously for 10 doses. First injection was 0.05 ml, other 9 were 0.1 each given every other day for 3 wks.	No evidence of dermal sensitization.	Test material contained beta-exotoxin.	Fisher and Rosner 1959
Suspension of Thuricide	Guinea Pig	Ten topical applications of powder administered on abraded skin every other day for 3 wks. First application was 25 mg, other 9 were 50 mg each.	Slight erythema and edema indicative of local irritation, but no evidence of any allergic response.	Test material contained beta-exotoxin.	Fisher and Rosner 1959
Suspension of Thuricide	Guinea Pig	Ten topical application to intact skin made in same manner as with abraded skin.	No evidence of any allergic response.	Test material contained beta-exotoxin.	Fisher and Rosner 1959

Appendix E (Concluded)

Formulation	Organism	Nature of Exposure	Effects	Comments	Reference
		Dermal Sensitization (Concluded)			
B.t. in Tween in Prophyllite dust	Guinea Pig	Groups of 6 male animals were sensitized by simultaneous s.c. and i.p injection of test material. Groups received total doses 0, 0.4, 8, 40, 200, or 400 mg. Challenge occurred 21 days later by aerosolization.	Deaths (6/6, 6/6, and 2/6 at levels of 400, 200, and 40 mg, respectively) occurred within 18 to 24 hrs after the sensitizing doses. No deaths following challenge doses were observed among the remaining animals.	Test material may have contained beta-exotoxin as a toxic agent.	Kimura 1970 in Forsberg et al. 1976
		Chronic Toxicity			
Biotrol	Rat	Groups of 25 male and 25 female animals were fed Biotrol (25 x 10^9 spores/g) in the diet at a 1% level for 2 yrs.	No statistical differences between test animals and control animals.	Test material may have contained beta-exotoxin as a toxic agent.	Barnes 1970 in Forsberg et al. 1976
Commercial manufactured products	Human	Eight men were exposed over a 7-mo period to whole fermentation broth, (300 ml to several thousand gals/day); moist bacterial cake (50 g to several thousand lbs/day); effluent (300 ml to several thousand gals/day); and final powder (10 g to several thousand lbs/day, containing up to 15 x 10^9 viable spores/g).	The workers were free of any complaints. Two individuals examined comprehensively were in excellent health and showed no evidence of acute or chronic toxicity of any kind.	--	Fisher and Rosner 1959

NA - data not available.

Appendix F

Summary of Bacillus thuringiensis Toxicity Data for Neurotoxicity and Behavior

Appendix F

Summary of Bacillus thuringiensis Toxicity Data for Neurotoxicity and Behavior

Formulation	Organism	Nature of Exposure	Time of Exposure	Effects	Comments	Reference
B.t. var. israelensis	Rat	Oral dose 2 x 10^9 spores/ml. Rats bled at 3, 7, 21 days	3 to 21 days	Feed consumption increased ($p < .05$) interaction of sex and treatment was significant ($p < .05$) for gain and efficiency.	No details or data given.	Abbott Laboratories Exp. Summary 912-1959
B.t. var. israelensis	Rat	Intratracheal instillation dose 0.8 x 10^9/ml	--	No abnormal behavior.	No details or data given.	Abbott Laboratories Exp. Summary 912-1979
B.t. var. israelensis	Rat	Acute oral 2.67 g/kg	14 days	No abnormal behavior.	No details or data given.	Abbott Laboratories Exp. Summary 912-1986

Source: Abbott 1986 (unpublished).

Appendix G

Summary of Bacillus thuringiensis Mutagenicity Data

Appendix G

Summary of Mutagenicity Data for Bacillus thuringiensis

Formulation	Organism	Nature of Exposure	Effects	Comments	Reference
Purified exotoxin from autoclaved supernatant of	Salmonella typhimurium	Bacterial strains TA1535, TA1537, TA1538, TA98, and TA100 were exposed to varied concs. (1,000 to 100,000 µl/plate) of exotoxin both with and without rat liver microsomal enzyme preparation.	Not mutagenic. No significant increase in the number of revertant colonies compared to the controls.	NA	Cantwell et al. 1982
Autoclaved supernatant of B.t. serotype 1, serotype 3, and purified exotoxin	Saccharomyces cerevisae, strains D4 and D5	Cells treated with test materials and examined for induction of mitotic gene conversion.	No indication of mutagenic activity.	NA	Linnainmaa et al. 1977
Beta-exotoxin (autoclaved supernatant of B.t. serotype 1, a producer of beta-exotoxin)	Human lymphocyte cultures	Cultures treated with 20% v/v conc. of supernatant. ASI.	Increased incidences of chromosome aberrations.	NA	Meretoja et al. 1977
Autoclaved supernatant, of B.t. serotype 3 (nonproducer)	Human lymphocyte cultures	Cultures treated with 20% v/v conc. of supernatant, ASI.	No increased incidence of chromosome aberrations.	NA	Meretoja et al. 1977

Appendix G (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	Reference
Autoclaved supernatant of *B.t.* serotype 1, serotype 3, and purified exotoxin	*Drosophila melanogaster*, fruit fly	Test materials fed to the fruit flies during entire period of development. Nondisjunction tests and sex-linked recessive tests performed.	Negative results in nondisjunction tests. Very slight but statistically significant ($p < 0.01$) increase in frequency of sex-linked recessive lethal mutations induced by autoclaved supernatant of serotype 1.	NA	Linnainmaa et al. 1977
Beta-exotoxin, delta-endotoxin protein	*Allium cepa* *A. sativum*, *Vicia faba*	Growing root meristems of the plants were exposed to 50 ppm solutions for 1 to 3 hrs, fixed, squashed and examined for clastogenic activity.	The beta-exotoxin caused clastogenesis in *A. sativum* only. The major aberrations were chromosome breaks, gaps, occasional chromatid gaps.	NA	Panda et al. 1979
Autoclaved supernatant from *B.t.* serotype 1	Rat	100%, 50%, and 5% conc. given in drinking water for 3 mos.	No increased incidence of chromosomal aberrations in bone marrow of animals administered up to 100%, but increased incidence of chromosomal aberrations in lymphocytes of animals fed 50% and 100% concs. of supernatant.	Beta-exotoxin	Meretoja et al. 1977
Autoclaved supernatant from *B.t.* serotype 1	Rat	100% v/v administered in drinking water for 6 mos. Bone marrow cells examined.	No increase in chromosome aberrations over the controls.	Beta-exotoxin	Kahkonen et al.

Appendix G (Concluded)

Formulation	Organism	Nature of Exposure	Effects	Comments	Reference
Beta-exotoxin from autoclaved suspension of B.t. serotype 1	Rat	Intraperitoneal (i.p.) lethal dose. Bone marrow cells examined.	No induction of chromosome aberrations.	NA	Meretoja et al. 1977
Autoclaved supernatant from B.t. serotype 1	Rat	I.P. injection of supernatant at a conc. of 50 mg/kg body wt given hourly for 9 hrs. Last injection included a sublethal dose (2.5 ml supernatant concentrated 10 x). Rats killed 12 hrs later. Bone marrow examined for sister chromatid exchanges.	Not mutagenic. No increase in sister chromatid exchanges over the controls.	NA	Kahkonen et al. 1979

NA = data not available.

Appendix H

Summary of Metabolism Studies with Bacillus thuringiensis

Appendix H

Summary of Metabolism Studies with _Bacillus thuringiensis_

Formulation	Organism	Dose	Comments	Reference
Beta-exotoxin, ^{32}P-labeled	Caterpillars (_Galleria mellonella_)	Caterpillars injected with 5.35 g (6.3 x 10^{-2} µCi) in 10 µl of water into hemolymph or administered orally.	Hemolymph was withdrawn at 20, 60, and 180 min after injection and oral administration. Distribution of radioactivity after injection was 83.3, 80.6, and 84.7% in the hemolymph at 20, 60, and 180 min. Of the remaining radioactivity, 6.4, 6.5, and 8.0% was in fat bodies; 3.6, 5.7, and 3.2% was intestine; 0.6, 0.8, and 0.6% was in Malpighian tubules; and 0.6, and 0.4% was in spinning glands after 20, 60, and 180 min. Injected material underwent essentially no degradation in the hemolymph. Material administered perorally was degraded in the intestine.	Vankova et al. 1974
Delta-exotoxin (derived from _B.t._ var. _kurstaki_, strain HD-1)	Lepidopteran (Bombyx mori; silkworm)	Crystals (2 mg/ml) were incubated for 30 min at 27 °C at pH values of 7.0 to 13.0 with and without gut juice protease from silkworm.	The original 120,000-dalton subunit of the crystal was degraded, in the presence of gut juice protease, to a toxic protein with a mol wt of 59,000 and to lower mol wt (less than 40,000) proteins that were not toxic to silkworms.	Tojo and Aizawa 1983

Appendix H (Concluded)

Formulation	Organism	Dose	Comments	Reference
Thuricide 16B	Fresh water clams, unspecified species	Surrounding 2,000-acre watershed treated at 4 BIU/acre (9.88 BIU/ha).	Clams contained viable spores 2 days after application, but none 30 days after application.	Buckner et al. 1974 in Forsberg et al. 1976
^{32}P-labeled "exotoxin"	Mice (female white mice, strain H)	Mice were administered labeled exotoxin (2.66 (2.66 C/500 g/animal) intraperitoneally.	Mice were sacrificed at various intervals up to 240 min after dosing. Excretion of exotoxin in the urine started immediately: at 20 min after dosing, 10% had been excreted; at 60 min, 20%. After 60 min, essentially no exotoxin was excreted in the urine. Exotoxin was rapidly dephosphorylated in the tissues, with rapid decrease in tissue concs. Tissue concs. were: 3.64 and 1.24 ppm, kidney; 0.46 and 0.15 ppm, intestine; 0.42 and 0.13 ppm, heart; 0.88 and 0.35 ppm, spleen; and 0.90 and 0.53 ppm, liver at 30 and 75 min after administration, respectively.	Sebesta and Horska 1973

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Carbaryl Summary

Major Trade Name:	Sevin
Chemical Name:	1-Naphthyl N-methylcarbamate
Major Forestry Application:	Insect control, especially the western spruce budworm

Detailed information on toxicology, environmental fate, and hazard assessment can be found in the body of this pesticide background statement. The purpose of this statement is to support USDA Forest Service risk assessments relating to the use of carbaryl in forest insect pest management in the National Forest System.

Toxicology

Most species of microorganism tested are adversely effected by carbaryl at sublethal concentrations although there is significant variability in the results of the tests. Similarly, there is wide variability in the results of toxicity in invertebrates; in earthworms, for example, LC_{50}'s (median lethal concentration) ranged from 3.1 to 6.3 ppm (parts per million) in one species to 200 to 800 ppm in another species. Carbaryl is moderately toxic to highly toxic in a wide variety of aquatic crustacea and toxic to many nontarget insects, including bees. LC_{50}'s for carbaryl for fish are generally in the range considered to be toxic (i.e., 1 to 10 ppm). The acute oral toxicity of carbaryl in birds ranges from slightly to moderately toxic.

The acute toxicity of carbaryl exposure to mammals is high by the oral route but very low by the dermal route of exposure. It is mildly toxic to guinea pigs exposed by inhalation. Carbaryl does not appear to be teratogenic or have significant reproductive effects. It does not seem to be carcinogenic although it has been shown to be weakly mutagenic in some mutagenicity test systems. Neurotoxic effects have been observed in some mammals.

Environmental Fate

Carbaryl absorbed by plants can be toxic to the plants and can be metabolized to several water-soluble compounds. The amount of carbaryl detected as residues in and on plants after application was highly variable in several studies. The amount of residue varied with the mode of application, the amount of carbaryl applied, the species of plant observed, and the amount of time between application and measurement of the residue.

Carbaryl has a moderate persistence in most soils. It sorbs physically to soil particles and can be degraded in soil. Carbaryl is stable in water that is acidic but will hydrolyze in water that is neutral or basic. Carbaryl is not expected to persist in aquatic environments.

Exposure and Hazard

Carbaryl, although widely used for forest insect pest management, is applied at application rates sufficiently low that humans and wildlife are not expected to be adversely affected. Carbaryl does not appear to be carcinogenic or to have adverse reproductive effects at the low exposures that would occur from its use by the USDA Forest Service.

1.0 General Information

Carbaryl is an insecticide that is also known primarily by the trade name Sevin in the United States. Other trade names include Carbacide®, Carpolin®, Denopton®, Dicarbam®, Hexavin®, Karbaspray®, Karbotox®, Mervin®, Nac®, Ravyon®, Septene®, Sevinox®, Tercyl®, and Tricarnam® (Hayes 1982, Meister Publishing Company 1984). Code designations for carbaryl have included ENT-23,969, Experimental Insecticide-7,744, OMS-29, UC 7744, and Union Carbide 7,744 (Hayes 1982, International Agency for Research on Cancer 1976). Carbaryl is produced in the United States by Union Carbide, Agricultural Products Company, Inc. It is also imported into this country from Israel, where it is produced by Makhteshim Chemical Works, Ltd. (U.S. Environmental Protection Agency undated). Carbaryl is marketed alone and as a combined formulation with other insecticides, such as lindane. There are a wide variety of carbaryl formulations. It is formulated in the form of baits, dusts, wettable powders, molasses dispersions, aqueous dispersions, granules, and oil suspensions (Kuhr and Dorough 1976, Meister Publishing Company 1984).

Carbaryl is a residual contact and stomach insecticide (Kuhr and Dorough 1976). In vertebrates, its toxic effects are primarily due to cholinesterase (ChE) inhibition (Kuhr and Dorough 1976). It is used primarily as a broad-spectrum insecticide but is also used specifically as an acaricide and molluscicide in some applications (International Agency for Research on Cancer 1976).

Carbaryl was first synthesized in 1953 by the Union Carbide Corporation and was originally designated as UC 7744 (Kuhr and Dorough 1976). It was introduced as an insecticide by Union Carbide in 1956 (Hayes 1982). By 1959, it had become a widely used insecticide in many parts of the world due to its relatively low toxicity to mammals, its environmental degradability, and its broad-spectrum insecticidal properties (Kuhr and Dorough 1976). In 1971, 55 million pounds of carbaryl were manufactured by Union Carbide.

The available body of scientific and popular literature on carbaryl, as well as on structurally related carbamate insecticides, is very extensive. This pesticide background statement will not attempt to review comprehensively all that has been written about carbaryl, but rather, it will provide a summary of what is known about its toxicity, health effects, environmental fate, and mode of action, particularly on the basis of studies that have been conducted during the past two decades. Numerous reviews are available on carbaryl as well as on the whole group of carbamate insecticides. Among these reviews are Hayes (1982), International Agency for Research on Cancer (1976), Kuhr and Dorough (1976), National Institute of Occupational Safety and Health (1976), and

U.S. Environmental Protection Agency (1983 and undated), and Cranmer (1986).

According to the U.S. Environmental Protection Agency (undated), there are insufficient data to provide a full assessment of the environmental fate of carbaryl. It appears to be stable under acidic conditions but decomposes rapidly under alkaline conditions. Carbaryl degrades in soil and is moderately mobile, particularly in the absence of organic carbon. It tends to bioaccumulate at low rates in aquatic organisms. It has a slight toxicity to most nontarget organisms with the exception of honey bees, and has average persistences of 3 to 4 days on plant foliage, 7 to 9 days in soils, and 1 to 5 days in water (Kuhr and Dorough 1976).

1.1 Normal Use Patterns

Carbaryl is widely used on a variety of agricultural crops, including cotton, corn, soybeans, stone fruits, table vegetables, bananas, pineapples, coffee, cacao, olives, rice, sugar cane, and a variety of other food, fiber, and forage crops (Kuhr and Dorough 1976). Other uses include insect control on forest and range lands; on animals such as livestock, poultry, and pets, and in buildings. In the United States, carbaryl is registered for the control of insect pests on more than 100 crops, such as citrus, pome, stone, and berry fruits; forage, field, and vegetable crops; nuts; lawns; forest and ornamental plants and trees; and range land. It is also registered for use as an insecticide on poultry and pets; and for indoor insect pest control (U.S. Environmental Protection Agency undated).

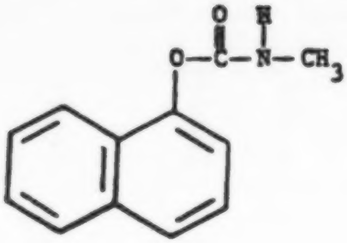
Carbaryl is usually applied at rates of 0.5 to 4.0 lb a.i./acre (pounds of active ingredient per acre) (0.56 to 4.48 kg/ha [kilograms per hectare]) or in concentrations of 0.5 to 1.5 lb a.i./100 gal (pounds of active ingredient per gallon) (60 to 180 g/100 l [grams per liter]) of water (Kuhr and Dorough 1976). The residue tolerances for carbaryl are relatively high compared to many insecticides (5 to 10 ppm) for many food crops. In addition, carbaryl is relatively short-lived in most environmental media. As a consequence of its short persistence and its high tolerances, carbaryl is often used on food crops within a few days of harvest.

Carbaryl formulations have been extensively used by the USDA Forest Service for control of the western spruce budworm, the pandora moth, various nursery and seed orchard insects, mountain pine beetle, and western pine beetle (Gross 1985). The most extensive application by the USDA Forest Service, however, is for control of the western spruce budworm. In 1983, more than 500,000 acres were treated in USDA Forest Service projects by aerial spray with a liquid carbaryl formulation at a rate of 1.0 lb. a.i./acre (1.12 kg/ha). Ground application of carbaryl for USDA Forest Service projects in that year amounted to 1.0 to 2.0 lb. a.i./acre (1.12 to

2.24 kg/ha) applied to approximately 165 acres. USDA Forest Service use of carbaryl in 1983 is summarized in table 1-1.

1.2 Chemical and Physical Properties

The active ingredient of carbaryl and its formulations is 1-naphthyl N-methylcarbamate, with the following chemical structure (Meister Publishing Company 1984):



The physical and chemical properties of carbaryl have been reviewed by a number of authors, including Hayes (1982), International Agency for Research on Cancer (1976), Kuhr and Dorough (1976), and U.S. Environmental Protection Agency (undated). Unless otherwise specifically noted, the following discussion of the physical and chemical properties of carbaryl is taken from these reviews.

Carbaryl has a molecular formula of $C_{12}H_{11}NO_2$ and a molecular weight of 201.2. In its pure form, it is a white crystalline material with a reported melting point of 287.6 to 293 °F (142 to 145 °C) and no odor. The density of carbaryl is 1.232 grams/milliliter (g/ml) at 68 °F (20 °C). It has a vapor pressure of less than 5×10^{-3} mmHg at 78.8 °F (26 °C).

In water, carbaryl has a solubility of 40 ppm at 86 °F (30 °C). It is soluble in most polar solvents and in petroleum oils has a solubility of approximately 5 percent. For this reason, several liquid formulations of carbaryl are in oil solvent, and carbaryl is often applied as a spray in a diesel oil carrier. Carbaryl is stable in acidic buffered aqueous solutions and hydrolyzed in alkaline buffered solutions (U.S. Environmental Protection Agency 1983). The half-life for hydrolysis of carbaryl under acidic conditions at a pH of 5 at 80.6 °F (27 °C) was calculated to be greater than 4.38 years based on estimated rate constants. Under alkaline conditions at pH of 9 at 80.6 °F (27 °C), however, the hydrolysis half-life of carbaryl was 3.6 hours based on second-order rate constants. In studies on the degradation of carbaryl in sterile pond water, the half-lives of carbaryl were reported to be 30 and 12 days, respectively, at pH values of 6.7 and 7.2. The primary product of carbaryl hydrolysis is 1-naphthol.

Table 1-1

USDA Forest Service Carbaryl Use 1983

Purpose	Active Ingredient per Acre (lb)		Acres Treated	Formulation	Application Method
	Range	Average			
		1983			
Western spruce budworm control	1.0	--[a]	501,994	Liquid	Aerial spray (1)
Pandora moth control	1.0	--	50	Liquid	Aerial spray (1)
Nursery insect control	1.0	--	45	Liquid	Ground (3)
Seed orchard insect control	2.0	--	30	Liquid	Ground (1)
Mountain pine beetle, spruce beetle, and western pine beetle	1.0	--	90 (8,795 trees)	Liquid	Ground (9)

[a] Data not available.

Source: Gross 1985

Carbaryl is subject to photolysis, with a reported half-life of 45 hours in distilled water at pH 5 when exposed to mid-day sunlight in June (U.S. Environmental Protection Agency 1983). Under natural environmental conditions, photolysis of carbaryl varies seasonally, with the rate during the spring and summer months being approximately four times that of the fall and winter months. In a solution of ethanol, carbaryl degraded into 1-naphthol and five unidentified ChE-inhibiting phenolic compounds (postulated to be polyhydroxylated naphthalenes) after 3 hours of exposure to ultraviolet light. Crosby et al. (cited as GS 0080-1066 in U.S. Environmental Protection Agency 1983) reported decomposition of carbaryl into one unidentified minor ChE-inhibiting compound following exposure to sunlight or to weak ultraviolet light.

2.0 Toxicology of Carbaryl

Carbaryl is a widely used insecticide that has a low toxicity to mammals and birds and that also presents a relatively low potential hazard to most nontarget organisms with the exception of honey bees (Kuhr and Dorough 1976). Table 2-1 summarizes the toxicity data that are available for mammals and birds for carbaryl formulations.

Carbaryl inhibits ChE inhibitor in acute exposures. It may have other modes of action under chronic exposure, or chronically exposed animals may exhibit toxic effects other than ChE inhibition that are due to metabolites of carbaryl (Hayes 1982).

Many of the studies of toxic effects of carbaryl deal with its efficacy in controlling a variety of actual or potential target insects and other invertebrates. The majority of these studies deal with the effects of carbaryl formulations on various species of lepidoptera, although some studies are concerned with flies, aphids, beetles, wasps, grasshoppers, ticks, and slugs. The results of some of these studies on different target organisms are briefly summarized in table 2-2 and will not be considered further in this pesticide background statement.

In the following discussion of the toxicity of carbaryl, mutagenicity studies are discussed in the section on mammalian toxicity regardless of the specific test system or organism that was used. This is because the ultimate purpose of such mutagenicity studies is to determine the potential mutagenic effects of the test compound in humans.

2.1 Microorganisms

Because of the widespread use of carbaryl in agricultural, forestry, and indoor applications, there has been considerable interest in the potential toxicity of carbaryl formulations to various microorganisms that are normal inhabitants of soil and water. Several studies have also been concerned with the toxicity of carbaryl to specific pathological microorganisms, including *Bacillus thuringiensis*, which is used as a bacterial insecticide, and several pathogenic fungi of arthropods. These studies, which are summarized in appendix A, indicate that carbaryl has a variable effect on microorganisms. However, most species tested show some form of adverse effect, in terms of growth or inhibition of a specific life-stage, at sublethal concentrations of carbaryl.

Studies with aquatic microorganisms have shown variability in toxic responses to carbaryl. Ukeles (1962, in Butler 1977 and in Ware and Roan 1970) cultured five species of marine phytoplankton for as long as 10 days. He reported total inhibition of growth in cultures of three species of phytoplankton exposed to carbaryl concentrations of 10 ppm, in cultures of

Table 2-1

Summary of Toxicological Properties of Carbaryl

1. Acute Oral Toxicity

Organism (Formulation)	Exposure	Adverse Effects
Rat (carbaryl)	Ingestion of a single dose (fasted rats).	LD_{50} - 246 mg/kg (female). LD_{50} - 283 mg/kg (male).
Mouse (carbaryl)	Ingestion of a single dose.	LD_{50} - 275 mg/kg
Rabbit (carbaryl)	Ingestion of a single dose.	LD_{50} - 710 mg/kg
Man (carbaryl)	Ingestion of a single dose.	Epigastric pain, sweating, lassitude and vomiting.
Hen (carbaryl)	Ingestion of a single dose.	LD_{50} - 248 mg/kg.
Redwing blackbirds (carbaryl)	Acute exposure.	LD_{50} - 56 mg/kg.
Bobwhite quail (carbaryl)	Ingestion of a single dose of 10 to 90 mg/kg.	No significant effects; no deaths.
Game birds (6 species) (several carbaryl formulations)	Ingestion of a single dose.	Pheasant LD_{50} - 707 mg/kg Pigeon LD_{50} - 3,000 mg/kg
Coturnix quail and mallards (carbaryl)	Ingestion of a single dose of 1,000 mg/kg.	Less than 5 micron reduction in egg shell thickness.

2. Acute Intraperitoneal Toxicity

Organism (Formulation)	Exposure	Adverse Effects
Rat (carbaryl)	Details not given.	LD_{50} - 200 mg/kg
Mouse (carbaryl)	Details not given.	LD_{50} - 25 to 29 mg/kg
Rabbit (carbaryl)	Details not given.	LD_{50} - 223 mg/kg

Table 2-1 (Continued)

3. Acute Dermal Toxicity

Organism (Formulation)	Exposure	Adverse Effects
Rat (carbaryl)	Details not given.	LD_{50} >4 g/kg
Japanese quail (5% carbaryl)	External dusting.	Slight reduction in plasma ChE.
Field populations of birds (carbaryl)	Field application 1 lb/acre (1.12 kg/ha) twice.	Decrease bird population, number of species and species diversity (1 study).
Field populations of birds (carbaryl)	Field application 1 lb/acre (1.12 kg/ha)	No significant decrease in bird numbers.
Field populations of birds (carbaryl)	Field application 2 lb/ac (2.24 kg/ha).	No abnormal behavior or ill birds; minor decrease in ChE levels.
Field populations of birds (carbaryl)	Field application 0.5 + 0.3 lb/ac (0.56 + 0.34 kg/ha).	No significant difference in ChE levels.
Field populations of birds (carbaryl)	Field application of 1 lb/ac (1.12 kg/ha).	No change bird activity but reduced food supplies.
Turkeys (carbaryl)	Extremely high application rate (14 X recommended).	16% mortality in 10 days; skin and fat tissue concs 1.7 and 3.6 ppm.

4. Acute Intravenous Toxicity

Organism (Formulation)	Exposure	Adverse Effects
Rat (carbaryl)	Details not given.	LD_{50} = 24 to 42 mg/kg.

Table 2-1 (Continued)

5. Eye Irritation

Organism (Formulation)	Exposure	Adverse Effects
Rabbit (carbaryl)	10% suspension in propylene glycol.	Mild injury.
Rabbit (carbaryl)	25% suspension in water.	No injury.
Rabbit (carbaryl)	50 mg of dust.	Mild corneal necrosis.

6. Inhalation Toxicity

Organism (Formulation)	Exposure	Adverse Effects
Guinea Pig (50% WP)	$390 mg/m^3$, for 4 hrs.	Nasal and ocular irritation.
Guinea Pig (85% WP)	$230 mg/m^3$, for 4 hrs.	Slight weight loss but regained their pretreatment weight by the end of 14-day observation period.
Man (85% WP)	6-hour exposure to a carbaryl sprayed room at the spray rate of $2 g/m^2$.	Skin rash in a sprayman whose back was splashed accidently with 5% carbaryl spray.

7. Subchronic Toxicity

Organism (Formulation)	Exposure	Adverse Effects
Rat (carbaryl)	Dietary feeding of carbaryl at 0, 1,500, 2,500 ppm for 96 days.	Increased kidney weight of females at both doses and cloudy swelling of kidney in females at 2,500 ppm.

Table 2-1 (Continued)

7. Subchronic Toxicity (Concluded)

Organism (Formulation)	Exposure	Adverse Effects
Man (carbaryl)	Ingestion of carbaryl daily for 6 wks at 0.06 mg/kg or 0.12 mg/kg.	0.12 mg/kg caused abnormality in kidney function.
Rat (carbaryl 85% WP)	10 mg/m^3, 7 hrs/day, 5 days/wk, 90 inhalation periods.	No mortality nor grossly visible injury was observed.
Japanese quail (carbaryl)	14 wks chronic dietary exposure.	Higher concs (>900 ppm) decreased body weight, increased kidney weight, slight decrease in egg production. >600 ppm males elevated brain dopamine levels.
White leghorn (Kaolin powder 50% Sevin)	60-day chronic dietary exposure.	100% mortality at 540 mg/kg/day for 35 days; after 3 days birds restless, diarrhea, decrease appetite, liver degeneration, spleen atrophy; enlarged gall bladder, degenerated ovaries.
Bobwhite quail (carbaryl)	7-15-25-day chronic dietary exposure.	Increased suscepti- bility to infections.

Table 2-1 (Continued)

8. Chronic Toxicity

Organism (Formulation)	Exposure	Adverse Effects
Rat (carbaryl)	Dietary feeding at 0, 20, 50, 100, 200, or 400 ppm for 2 yrs.	NOEL - 200 ppm. In 400 ppm group, cloudy swelling of hepatic cords after 2-year feeding.
Rat (carbaryl)	Rats were administered carbaryl orally at 7, 14, or 70 mg/kg for 1 yr (method of administration is not clear).	NOEL was not established. Thyroid, gonadal and pituitary function were impaired at all dose levels.

9. Reproductive and Teratogenic Effects

Organism (Formulation)	Exposure	Adverse Effects
Rat (carbaryl)	Daily peroral administration of 2, 5, or 15 mg/kg/day for 1 year prior to mating.	The NOEL for reproductive effects = 2 mg/kg.
Rat (carbaryl, 99.8% pure)	Dietary feeding of carbaryl at 0, 2.5, or 10 mg/kg/day for three generations.	No reproductive effects at all dose levels.
Rat (carbaryl)	Dietary feeding of carbaryl at 0, 2,000, 5,000, or 10,000 ppm (equivalent to 100, 250, or 500 mg/kg/day) for three generations.	Reproductive effects at all dose levels.

Table 2-1 (Continued)

9. Reproductive and Teratogenic Effects (Continued)

Organism (Formulation)	Exposure	Adverse Effects
Gerbil (carbaryl)	Dietary feeding of carbaryl at 0, 2,000, 4,000, 6,000, or 10,000 ppm for three generations.	The NOEL for reproductive effects = 4,000 ppm.
Rat (carbaryl) 99.8% pure)	Dietary feeding of carbaryl at 0, 20, 100, or 500 mg/kg from pregnancy through weaning.	Not teratogenic.
Monkey (carbaryl)	Gastric intubation of 2 or 20 mg/kg throughout gestation period.	Not teratogenic, but rate of abortion was high.
Mouse (carbaryl, 99% pure)	Daily administration of carbaryl at 0, 100, or 150 mg/kg by gavage or dietary feeding of carbaryl at 5,600 ppm from days 6 through 15 of gestation.	Not teratogenic.
Hamster (carbaryl)	Daily oral intubation at 125 or 250 mg/kg from days 12 through 20 of gestation.	Not teratogenic.
Dog (carbaryl)	Ingestion of carbaryl throughout gestation at 3.125, 6.25, 12.5, 25, or 50 mg/kg.	The NOEL for teratogenic effects = 3.125 mg/kg. Teratogenic at 6.25 mg/kg and higher doses, however, dose-response relationship was not established.

Table 2-1 (Continued)

9. Reproductive and Teratogenic Effects (Continued)

Organism (Formulation)	Exposure	Adverse Effects
Guinea Pig (carbaryl)	Daily oral intubation of carbaryl at 200 mg/kg or daily dietary inclusion of carbaryl at 300 mg/kg for three generations.	Not teratogenic.
Guinea Pig (carbaryl)	Daily oral intubation at 300 mg/kg from days 12 through 20 of gestation.	Teratogenic. There was increased maternal and fetal mortality.
Rabbit (carbaryl)	Daily oral intubation at 50 to 200 mg/kg from days 5 through 15 of gestation.	Not teratogenic.
Rabbit (carbaryl 99% pure)	Daily oral intubation at 0, 150, or 200 mg/kg from days 6 through 18 of gestation.	Teratogenic.
Mallard eggs (carbaryl)	Eggs dipped on 3rd day of incubation.	LC_{50} - 216 lb/ac (242 kg/ha) (aqueous) LC_{50} - 24 lb/ac (26.9 kg/ha)(oil); reduced growth; abnormal survivors; LD_{50} - 118 g/egg
Chicken eggs (carbaryl)	No details given.	1 mg/egg malformation of chickens.
Chicken eggs (carbaryl)	Inject dose into yolk.	Body weight decrease leg and body length decrease, abnormal feathers; increase mortality 10 mg/egg after exposure; up to 12 days deformed embryos.

Table 2-1 (Continued)

9. Reproductive and Teratogenic Effects (Concluded)

Organism (Formulation)	Exposure	Adverse Effects
Chicken (carbaryl)	Injection into allantoic cavities of embryos.	LD_{50} = 3.44 mg/embryo.
Japanese quail (carbaryl)	Injection into air space of embryo and egg.	Embryonic weights altered, 10% defective development.
Birds (carbaryl)	Field application 1 lb/ac (1.12 kg/ha).	100% nest success; no nest mortality; no nestling weight loss.

10. Carcinogenicity

Organism (Formulation)	Exposure	Adverse Effects
Rat (carbaryl)	Subcutaneous implantation of 20 mg of carbaryl in paraffin capsule and kept for 22 mos.	Inconclusive.
Rat (carbaryl)	Daily intubation of carbaryl at 30 mg/kg for 22 mos.	Inconclusive.
Rat (carbaryl)	Dietary feeding of carbaryl at 0, 2, 4, 8, or 16 mg/kg for 2 yrs.	Not carcinogenic.
Rat (carbaryl)	Pregnant rats received a total dose of 300 mg carbaryl by gavage over 10-day period with and without sodium nitrite.	Not carcinogenic.

Table 2-1 (Continued)

10. Carcinogenicity (Concluded)

Organism (Formulation)	Exposure	Adverse Effects
Mouse (carbaryl)	Daily oral administration of carbaryl by stomach tube at 4.64 mg/kg from 1 wk of age up to 4 weeks of age followed by 14 mg/kg until 78 wks of age.	Not carcinogenic.
Mouse (carbaryl)	Single subcutaneous injections of carbaryl at 100 mg/kg from 4 wks of age up to 78 wks of age.	Not carcinogenic.
Mouse (carbaryl)	Subcutaneous injections of carbaryl at 10 mg/animal weekly over 5-mo period.	Not carcinogenic.
Mouse (carbaryl)	Twelve intraperitoneal injections of carbaryl over 4 week period. Total dose of carbaryl was 6 mg/animals. The animals were kept 20 wks treatment.	Inconclusive.

11. Neurotoxicity

Organism (Formulation)	Exposure	Adverse Effects
Rat (carbaryl, 99.9% pure)	Intraperitoneal (ip) and intramuscular (im) injections of carbaryl at 1, 3, 5, or 10 mg/kg, or 2 times per wk for 6 mos.	Response rate decreased following 3, 5, or 10 mg/kg ip injections and 5 or 10 mg/kg im injections.

Table 2-1 (Continued)

11. Neurotoxicity (Concluded)

Organism (Formulation)	Exposure	Adverse Effects
Rat (carbaryl)	Dietary feeding of carbaryl at 100, 500, 2,500 ppm for 35 days.	Ability to avoid electric punishment decreased.
Rat (carbaryl)	Dietary feeding of carbaryl at 100 or 200 ppm for 5 days.	Affected: 1. Process of learning and performance of learned task. 2. Electroencephalographic pattern. 3. Inhibition of brain acetylcholinesterase (AChE) activity. 4. Functional deviations of the central nervous system.
Monkey (carbaryl)	Details not given.	Number of errors and time required to finish acquisition task increased.
Swine	Single oral administration at 1 or 2 g/kg.	Caused salivation, central-nervous system depression, muscle tremors, and cyanosis.
Swine	Dietary feeding at 150 to 300 ppm/day for up to 12 wks.	Caused paraplegia and prostration.
Chicken (carbaryl)	Acute subcutaneous injection.	Neurotoxic effects lasting 24 days; leg weakness and paralysis.

Table 2-1 (Continued)

12. Mutagenicity

Organism (Formulation)	Exposure	Adverse Effects
Barley seeds *Hordeum vulgare* (carbaryl)	1,000 ppm for 12 hrs.	Mutagenic. (Chromosomal aberrations).
Human embryonic fibro blasts (carbaryl, 84% pure)	Dose levels were 20 to 80 μ/ml culture medium.	Mutagenic. (Chromosomal spindle fibre formation was inhibited).
Salmonella typhimurium. LT 12 (carbaryl)	A range of unspecified doses.	Not mutagenic.
S. typhimurium 4 strains (carbaryl)	A range of unspecified doses, with or without microsomal system.	Not mutagenic.
Escherichia coli (carbaryl)	A range of unspecified doses.	Not mutagenic.
Saccharomyces cerevisiae (carbaryl)	A range of unspecified doses.	Not mutagenic.
Drosophila melanogaster (carbaryl, 85% pure)	A range of unspecified doses.	Not mutagenic.
Rat (carbaryl)	Dominant lethal test was conducted by dietary feeding of carbaryl up to 200 mg/kg/day or oral intubation of carbaryl up to 100 mg/kg/day until 32 wks of age.	Not mutagenic.
Mouse (carbaryl)	Dominant lethal test was conducted by 5 oral treatments of carbaryl at 50 or 1,000 mg/kg.	Not mutagenic.

Table 2-1 (Concluded)

12. Mutagenicity (Concluded)

Organism (Formulation)	Exposure	Adverse Effects
Mouse (carbaryl)	*In vivo* cytogenetic testing in which bone marrow was examined from mice treatd with carbaryl at 146 mg/kg.	Not mutagenic.
Mouse (carbaryl)	Host-mediated assay in which tester strain was inoculated into mice treated with carbaryl at 483 mg/kg.	Not mutagenic.

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Table 2-2

Effects of Carbaryl on Target Organisms

Organism	Formulation	Effects	Reference
Lepidoptera			
Western Spruce Budworm, *Choristoneura occidentalis*	Carbaryl (Sevin-4-oil)	Carbaryl applied to 6th instar larvae gave a 7-day contact LD_{50} equivalent to 0.38 lb/acre (0.427 kg/ha) and an LD_{90} equivalent to 1.06 lb/acre (1.19 kg/ha). Carbaryl applied to leaves fed to 6th instar larvae gave a 5-day LD_{50} equivalent to 0.11 lb/acre (0.126 kg/ha) and an LD_{90} equivalent to 0.41 lb/acre (0.462 kg/ha).	Robertson and Rappaport 1979
Western spruce Budworm, *Choristoneura occidentalis*	Carbaryl Sevin XLR and Sevin-4-oil)	Seven-day contact LD_{50}'s were 64.7 and 239 g/ha for Sevin-4-oil and Sevin XLR, respectively. The 7-day oral toxicity for carbaryl was reported as LD_{50} - 35.6 ppm.	Robertson and Harverty 1981
Budworms, four species of *Choristoneura*	Carbaryl	The LD_{50}'s ranged from 3.65 to 109 µg/g body weight and the LD_{90}'s from 12.1 to 791 µg/g body weight.	Robertson et al. 1978

Table 2-2 (Continued)

Organism	Formulation	Effects	Reference
Gypsy Moth, Lymantria dispar	Carbaryl (Sevin-4-oil)	Aerial application at a rate of 1 lb/acre (1.12 kg/ha) resulted in 99% reduction in moth population and a reduced defoliation, although some of the moth mortality was due to an outbreak of NPV.	Reardon et al. 1979
Gypsy Moth, Porthetria dispar	Carbaryl	The 48-hr contact LD_{50}'s to larve were 0.057, 0.072, 0.234, and 1.07 µg/larvae for the 2nd, 3rd, 4th, and 5th instars, respectively.	Ahmad and Forgash 1975
Gypsy Moth, Porthetriah (Lymantria) dispar	Carbaryl (Sevimol)	Application of 1.0 and 0.5 lb a.i./10 gal. to apple trees resulted in significantly reduced numbers of larvae compared to controls 2 and 15 days after treatment.	Doane and Dunbar 1973
Douglas-Fir Tussock Moth Orgyia pseudotsugata	Carbaryl	Almost half of the sibling groups within a single population showed significantly different responses to carbaryl, indicating the potential value of genetic assays in pre-spray population surveys.	Stock and Robertson 1979 1979

Table 2-2 (Continued)

Organism	Formulation	Effects	Reference
Douglas-Fir Tussock *Orgyia pseudotsugata*	Carbaryl (Sevin-4-oil and Sevin liquid)	Contact LD_{50}'s (Sevin-4-oil) were 6.30, 10.5, 24.5, 93.8 49.7, and 41.3 g/ha for instars 1, 2, 3, 4, 5, and 6, respectively; LD_{90}'s were 53.9, 50.4, 46.9, 378, 162, and 216 g/ha for instars 1, 2, 3, 4, 5, and 6, respectively. Oral LD_{50}'s (Sevin liquid) were 31.8, 42.8, 33.4, 44.1, 24.1, and 5.04 ppm for instars 1, 2, 3, 4, 5, and 6, respectively; LD_{90}'s were 52.5, 68.1, 55.7, 116, 139, and 304 ppm for instars 1, 2, 3, 4, 5, and 6, respectively.	Robertson and Boelter 1979a
Tufted Apple Budmoth, *Platynota ideausalis*	Carbaryl 50 WP	Carbaryl at a conconcentration 435 g a.i. in 378 l was applied to apple trees at a rate of 945 l/ha (equivalent to 0.97 lb/acre or 1.089 kg/ha). Adults were exposed to treated trees in cages at 1, 4, 8, and 15 days after application. Adult mortalities were significantly ($p = 0.05$) greater than controls following 3 days exposure to material weathered for 8 days, but did not differ	Rock and Shaltout 1983

Table 2-2 (Continued)

Organism	Formulation	Effects	Reference
Tufted Apple Budmot (Concluded)		from controls for material weathered for 15 days. For larvae exposed to the same material in the laboratory, mortalities for both 1 and 7 day-old larvae were significantly ($p = 0.05$) greater than controls for material weathered 8 days and for 1 day-old larvae for material weathered for 15 days. There was not significant increase in egg mortality.	
Southern Pine Coneworm, *Dioryctria amatella*	Carbaryl	The contact 7-day LD_{50} to 5th instar larvae was greater than 200 μg/g.	DeBarr and Fedde 1980
Forest Tent Caterpillar, *Malacosoma disstria*	Carbaryl	Topical application to 4th instar larvae resulted in an LD_{50} of 2.31 μg/g and an LD_{90} of 6.59 μg/g.	Lyon et al. 1971
Elm Spanworm, *Ennomos subsignarius*	Carbaryl (Sevimol)	Application of 1.0 and 0.5 lb a.i./10 gal to apple trees resulted in significantly reduced numbers of larvae compared to controls 2 and 15 days after treatment.	Doane and Dunbar 1973

Table 2-2 (Continued)

Organism	Formulation	Effects	Reference
Bertha Armyworm, Mamestra configurata	Carbaryl	A solution of 0.01% carbaryl resulted in 10% mortality to 3rd instar larvae after 18 hrs of exposure.	Harris and Turnbull 1975
Fall Armyworm, Spodoptera frugiperda	Carbaryl	Carbaryl, applied to coastal bermuda grass at 1 and 2 lb/acre (1.12 and 2.24 kg/ha), was reported ineffective for control of armyworm. Maximum reported mortality rate was 21% control 1 day after application of 1 lb/acre.	Sheets et al. 1982
European Skipper, Thymelicus lineola	Carbaryl (Sevin 85W)	Carbaryl applied at 0.45, 0.9, and 134 lb a.i./acre (0.5, 1.0, and 1.5 kg/ha) resulted in reduced mean larval densities (105, 15, and 15/m^2) after 11 days compared to 130/m^2 for controls in 1975. In 1976, carbaryl applied at 0.22, 0.45, and 0.9 lb a.i./acre (0.25, 0.5, and 1.0 kg/ha) resulted in reduced mean larval densities (8, 7, and 2/m^2) after 15 days compared to 87/m^2 for controls.	Letendre and McNeil 1979

Table 2-2 (Continued)

Organism	Formulation	Effects	Reference
European Skipper (Concluded)		All application rates gave significant protection against defoliation at time of harvest of timothy crops for both years.	
Leafroller, *Platynota flavedana*	Carbaryl 50 WP	Larvae dipped for 10 sec. in a solution of 1 lb a.i./100 gal had mortality rate of 55%; larvae fed treated foliage showed a 50% mortality rate after 72 hrs feeding, and egg mortality was 20%.	Bobb 1972
Green-horned Caterpillar *Melanitis leda ismena*	Carbaryl	Carbaryl was applied at concs. of 0.05 and 0.1% to runoff to rice leaves and weathered 6 and 8 days, respectively. The leaves were fed to fasted larvae and time required to provide 50% mortality of larvae was reported (LT_{50}). LT_{50}'s of 46.78 and 40.02 hrs were reported for application of 0.05 and 0.1% solutions, respectively.	Chaturvendi and Mathur 1984

Table 2-2 (Continued)

Organism	Formulation	Effects	Reference
Spotted Bollworm, *Earias vitella*	Carbaryl (Sevin 50 WP)	The corrected mortalities of adult worms were reported to be 68.51 and 21.11% 48 hrs after exposure to plants treated with a 0.2% solution and allowed to weather for 1 and 5 days, respectively.	Patil and Pokharkar 1981
Tomato Hornworm, *Manduca quinquemaculata*	Carbaryl	Topical application to 3rd instar larvae resulted in an LD_{50} equivalent to a 50% solution. Formulated material (85% WP) applied to tobacco leaves resulted in 3rd instar larvae mortalities of 75 and 100% at application rates equivalent to 0.6 and 1.0 lb a.i./acre (0.672 and 1.12 kg/ha), respectively.	Harris ert al. 1983
Tobacco Stem Borer, *Scrobipalpa heliopa*	Carbaryl 50% WP	Residual contact toxicity was with leaves from seedlings treated with a solution of 2.3 mg/l. Percent mortality after 24 hrs was determined to be 100% for leaves weathered 0 to 4 days, and 93.3, 66.7, 20.0, 6.7, and 0% leaves weathered for 5, 6, 7, 8, and 9 days, respectively.	Rao and Das 1980

Table 2-2 (Continued)

Organism	Formulation	Effects	Reference
Diptera			
Melon Fruitfly, *Dacus cucurbitae*	Carbaryl 50 WP	Contact toxicity to adults was by dry film technique and a 24-hr LC_{50} was reported as 5.678 ppm (0.0005678% conc.).	Gupta and Verma 1979
Homoptera			
Eastern Spruce Gall Aphid, *Adelges abietis*	Carbaryl 80 S	Application of full cover spray at 1 lb a.i./100 gal resulted in significant ($p = 0.05$) reduction in galls/tree (0.5) compared to controls (40.8) after 7 wks and a significant ($p = 0.05$) reduction in the numbers of nymphs/twig (7.6) compared to controls (78.7) after 23 wks.	Cameron et al. 1973
Green Peach Aphid, *Myzus persicae*	Carbaryl	Topical application resulted in 24-hr LD_{50}'s of 0.013 µg/insect (26.09 µg/g).	Lecrone and Smilowitz 1980

Table 2-2 (Continued)

Organism	Formulation	Effects	Reference
Brown Planthopper _Nilaparvata lugens_	Carbaryl 80% EC	Carbaryl was applied three times at a rate of 0.67 lb a.i./acre (0.75 kg/ha) to rice at approximately 2-wk intervals. There was no significant difference in populations of planthoppers or their predators between experimental and control plots.	Reissig et al. 1982
Brown Planthopper _Nilaparvata lugens_	Carbaryl	Third instar nymphs were exposed for 48 hrs to rice plants that had been treated with 8 ml of an 0.04% solution of carbaryl and allowed to weather up to 14 days. Percent mortalities 100, 100, 90, 80, 46.6, 26.7, and 10.8 on days 2, 4, 6, 8, 19, 12, and 14, respectively.	Koshiya et al. 1980
Hemiptera			
Leaffooted Pine Seedbug _Leptoglossus corculus_	Carbaryl	Topical application to 2-nd stage nymphs resulted in a 72-hr LD_{50} of 5.8 μg/g body weight and LD_{90} of 11 μg/g.	DeBarr and Nord 1978

Table 2-2 (Continued)

Organism	Formulation	Effects	Reference
Coleoptera			
Spruce Beetle, *Dendroctonus rufipennis*	Carbaryl (Sevin)	Topical application of 0.25 µl in acetone to the ventral thorax surface gave a 72-hr LD_{50} greater than 150 µg/g body weight.	Werner et al. 1983
Black Pine Weevils *Otiorhynchus sulcatus*	Carbaryl	Carbaryl had a low contact toxicity to adult weevils, with a maximum of 20% mortality 72 hrs after exposure to a 0.55 solution. No mortality was observed among adults allowed to feed for 72 hrs on foliage or waxed paper with a maximum conc. of 0.1%	Nielsen 1983
Elm Leaf Beetle *Pyrrhalta luteola*	Carbaryl 50% WP	Foliar spray of 1 lb a.i./100 gal to elms to runoff resulted in significant decrease in the mean number of adults, eggs, and larvae on tree branches during a 6-wk observation period.	Brewer 1973

Table 2-2 (Continued)

Organism	Formulation	Effects	Reference
Japanese Beetles *Popilla japonica*	Carbaryl	Carbaryl was applied to soybeans at rates of 4, 8, and 16 oz a.i./100 gal to runoff. Dried leaves were fed to field-collected adults after as long as 17 days weathering. In an initial study, mortalities after 72 hrs feeding were 100% for all three concs. after 1 to 2 days weathering, 95% for 4 oz. concs., and 100% for 8 and 16 oz. concs. after 3 to 4 days, and no mortality after 6 to 7 days in any group. In the second study, maximum 48-hr mortalities were 14, 40, and 89% for 4, 8, and 16 oz. treatments after 10, 12, and 17 days of weathering, respectively.	Lawrence et al. 1973
Mexican Bean Beetle, *Epilachna varivestris*	Carbaryl	The 48-hr contact toxicities for 1st instar larvae were reported as LD_{50} = 4.28 mg a.i./l and as LD_{95} = 7.66 mg a.i./l.	McClanahan 1981

Table 2-2 (Continued)

Organism	Formulation	Effects	Reference
Stem Weevil, *Cylindrocopturus adspersus*	Carbaryl	Applied at a rate of 2 lb a.i./acre (2.24 kg/ha), carbaryl reduced the mean numbers of larvae/sunflower stalk and the mean percent of parasitization/stem, but these reductions were not statistically.	Charlet and Oseto 1983
Bean Leaf Beetle, *Ceratoma trifurcata*	Carbaryl	The 24-hr contact LD_{50} was 3.8 μg/g body weight.	Chiu and Bass 1977
Lesser Mealworm, *Alphitobius diapernius*	Carbaryl 80 WP	The LD_{50}'s for topical application were reported to be 128.33 μg/g for adults and 56.83 μg/g for late instar larvae.	Vaughan and Turner 1984
Hymenoptera			
Western Yellowjacket, *Vespula pensylvanica*	Carbaryl	Mortalities 48 hrs after topical application were 20, 80, and 100% at concs. of 0.05, 0.1, and 0.5%, respectively.	Johansen and Davis 1972

Table 2-2 (Continued)

Organism	Formulation	Effects	Reference
Orthoptera			
Two-striped Grasshopper Melanoplus bivittatus	Carbaryl	The oral toxicities to 5th instar nymphs 24 hrs after ingestion of treated leaf discs were reported as LD_{50} = 23.18 μg/g and LD_{95} = 131.14 μg/g.	McDonald 1967
Migratory Grasshopper Melanoplus sanguinipes	Carbaryl	The oral toxicities to 5th instar nymphs 24 hrs after ingestion of treated leaf discs were reported as LD_{50} = 10.68 μg/g and LD_{95} = 26.40 μg/g.	McDonald 1967
Clear-winged Grasshopper Camnula pellucida	Carbaryl	The oral toxicities to 5th instar nymphys 24 hrs after ingestion of treated leaf discs were reported as LD_{50} = 15.37 μg/g and LD_{95} = 42.90 μg/g.	McDonald 1967
Acarina			
Dog Tick, Rhipicephalus sanguineus	Carbaryl 85% WP	Three-wk-old larvae were dipped in carbaryl solution. The 66-hr LC_{50} reported was 0.042% (420 ppm).	Crampton and Gichanga 1979

Table 2-2 (Concluded)

Organism	Formulation	Effects	Reference
Gastropoda			
Terrestrial Slugs, *Doroceras panormitanum* and *D. reticulatum*	Carbaryl	Carbaryl had a high toxicity to slugs. Applied as spray at 10 lb/acre (12.2 kg/ha) it killed 30% adults and 56% eggs of *D. panormitanum* and 40% adults and 67% eggs of *D. reticulatum*, respectively within 10 days. As a bait with 3.2% a.i., it killed 45% adults and 20% juveniles of *D. panormitanum* and 38% adults and 13% juveniles of *D. reticulatum*, respectively.	Van der Gulik and Springett 1980.

one species exposed to concentrations of 1 ppm, and in cultures of still another species exposed to concentrations of less than 0.1 ppm. Butler et al. (1975) reported that population growth in cultures of planktonic algae did not differ significantly from that of controls at concentrations of up to 1 ppm carbaryl. There was some inhibition of growth in cultures exposed to a concentration of 10 ppm carbaryl. There was no growth in 23 percent of the replicate cultures at a concentration of 25 ppm. Bringmann and Kuhn (1978) reported a toxicity threshold of 0.033 ppm carbaryl for inhibition of cell growth in the blue-green alga, Microcystis aeruginosa after 8 days of incubation. In the freshwater green alga, Scenedesmus quadricaudata, however, these same authors reported a toxicity threshold of 1.35 ppm carbaryl for inhibition of cell growth. Stadnyk et al. (1971) observed stimulated population growth in terms of cell numbers, carbon assimilation, and biomass during 6 days of culturing Scenedesmus quadicaudata with concentrations of up to 1.0 ppm carbaryl. Using the same species, Lejczak (1977) reported that 10 days of exposure to 0.1 ppm carbaryl resulted in a decrease in chlorophyll concentrations, and that increasing the concentration of carbaryl to 1,000 ppm (maximum concentration tested) resulted in decreasing overall chlorophyll contents of the algae. Maly and Ruber (1983) exposed 5 species of salt marsh algae to carbaryl. They reported that 48 hours of exposure to all but one (Gonyaulax) of the 5 algae resulted in significant ($p < 0.01$) inhibition of growth rates at concentrations of 1 ppm carbaryl or more during the first 48 hours of exposure, and at higher concentrations during the second 48 hours. Ramachandran et al. (1980) reported decreases in the growth rate (reduced rate of cell division) of the marine diatom (Coscinodiscus concinnus) when exposed for 5 days to concentrations of 1 ppb carbaryl or more. Growth rates were 91, 82, 80, 77, 64, and 54 percent that of controls at carbaryl concentrations of 1, 5, 10, 20, 40, and 50 ppb, respectively.

Several studies have examined the toxic effects of carbaryl on protozoa with varying results. Prescott et al. (1977) reported 59 percent reduction of growth and reproduction compared to controls among the free-living amoeba, Acanthamoeba castellanii, after 6 days exposure to 10 ppm carbaryl. In a protozoan culture dominated by a ciliate Euplotes sp., Weber et al. (1982) reported mean mortalities of 50 percent or more after 24 hours of exposure to a concentration of 1 ppm carbaryl, the lowest concentration tested. They reported higher mortalities (90 percent or more) in cultures exposed to concentrations of 6, 8, and 10 ppm carbaryl. In another study, with the ciliate Paramecium caudatum, Lejczak (1977) reported LC_{50}'s of 7.9, 12.4, and 10.2 ppm for 24, 48, and 96 hours of exposure, respectively.

Two studies have been reported on toxic effects of carbaryl on bacteria of the genus Bacillus. DeGiovanni-Donnelly et al. (1968) reported 50 percent inhibition of growth in cultures of B. subtilis after 6 hours of exposure to a concentration of 0.07 percent carbaryl compared to control cultures. Chen et al. (1974) reported that application of a carbaryl formulation at concentration of 0.47 g a.i./100 ml (approximately 0.5%) had no significant effect on viability of spores from one commercial formulation of insecticide derived from B. thuringiensis, but did cause significant time-related decreases in spore count for spores from a second formulation. On the other hand, Lejczak (1977) reported increases in dry weight compared to controls of the filamentous bacterium, (Sphaerotilus natans), following 72 hours of exposure to concentrations of 1 to 10,000 ppm carbaryl.

Several studies with cultures of fungus indicate that germination of the spores of fungi is not as sensitive to carbaryl as are other processes. Based on studies in which laboratory cultures of the fungus Trichoderma viride were incubated for as long as 14 days, Baicu (1982) reported that concentrations of 2,000 ppm carbaryl did not inhibit the germination of fungal spores, but that subsequent formation of spores was inhibited and the colonies exposed to carbaryl turned white. Hall (1981) cultured the entomopathogenic (i.e., pathogenic to insects) fungus Verticillium lecani for as long as 10 days in concentrations of 150 to 15,000 ppm carbaryl. Mycelial growth was inhibited at all concentrations tested, with 29 percent inhibition of growth at a concentration of 150 ppm and with 62 percent inhibition of growth at a concentration of 15,000 ppm concentration. In this study, germination of spores was reported following overnight exposure to carbaryl. Germination was not inhibited following exposure to 150 or to 1,500 ppm, and although germination was not quantified following exposure to 15,000 ppm, germination was observed at a concentration of 15,000 ppm. Merriam and Axtell (1983) observed the toxic effects of carbaryl on mycelial growth and the formation of zoospores in two isolates of another entomopathogenic fungus, Lagenidium giganteum. Among cultures from these two isolates, there was 50 percent inhibition of mycelial growth after seven days at concentrations of 43.7 and 39.4 ppm for isolates from North Carolina and from Louisiana, respectively. The maximum concentrations of carbaryl at which zoospores were produced were 5.1 and 12.6 ppm for the North Carolina and the Louisiana isolates, respectively.

Several studies that addressed toxic effects of carbaryl to microorganisms are described in appendix A. Baur and Misra (1970) reported that carbaryl concentrations of 100 ppm or more significantly reduced populations of bacteria in soil cultures only at 1 and 50 days of incubation, that actinomycetes were reduced by concentrations as low as 1 ppm, that the highest dose of carbaryl (5,000 ppm), had only a slight inhibitory effect on fungal populations, and that populations of Azotobacter sp. were inhibited only during the first day of exposure to carbaryl. Murray and Guthrie (1980) reported that carbaryl, applied to fresh water bacterial populations at a concentration of 5 ppm, resulted in a stimulation of bacterial metabolic rate, as did the principal degradation product of carbaryl, 1-naphthol.

2.2 Invertebrates

The potential toxicity of carbaryl to invertebrate organisms is of particular concern due to its widespread use to control a variety of target invertebrates and its toxicity to honey bees. Many of the target organisms for carbaryl applications are prey species for other members of the food chain.

Studies of the toxicity of carbaryl and its formulations to nontarget invertebrate organisms have included a variety of taxa, including pelycopod molluscs, oligochaete annelids, mites, crustaceans, and insects. These studies have primarily concentrated on species of beneficial organisms, organisms most likely to be exposed to carbaryl during commercial application, or on organisms that are particularly sensitive to environmental change. Studies summarized here are described in more detail in appendix B of this pesticide background statement.

Many of the available studies of the toxic effects of carbaryl in invertebrates have been conducted in controlled laboratory environments. As a consequence of the laboratory environment and the experimental designs employed, the results do not necessarily reflect the potential effects of carbaryl in a natural environment. For example, in the laboratory, aquatic invertebrates were often kept up to 4 days under static conditions. By contrast, in natural aquatic environments, exposure to carbaryl and other artificial chemicals is usually a transient phenomenon whose duration depends on the rate of stream flow or on the rate of decomposition of the chemical. Additionally, in natural systems, interactions with other man-made and naturally occurring chemicals, and changes in environmental factors, may affect assimilation and metabolism of the pesticide by invertebrates and microorganisms. These interactions could alter the tolerance levels of the organisms to the pesticide.

In most studies with pelycopod molluscs (clams, mussels, and oysters), carbaryl appears to be slightly to moderately toxic. Lethal effects, particularly among adults, have been reported for the most part, at concentrations above 1 ppm, and in some cases above 120 ppm. In several studies, concentrations of less than 1 ppm had some deleterious effects among larval stages, such as reducing development. Median tolerance limits (TL_{50}'s) for a wettable powder formulation of carbaryl in the bent-nosed clam were reported to be 27.5 and 17.0 ppm for 48 and 96 hours of exposure, respectively (Armstrong and Milleman 1974a). Histopathological evidence of epithelial tissue necrosis was observed among exposed individuals. Chaiyarach et al. (1975) reported 48 and 96 hour TL_{50}'s of 1,860 and 125 ppm, respectively, for clams exposed to an 80 percent wettable powder formulation of carbaryl. In cockle clams, 96 hour TL_{50}'s were 3.75 ppm for juveniles exposed to carbaryl and 2.7 ppm for juveniles exposed to the principal metabolite of carbaryl hydrolysis, 1-naphthol (Butler et al. 1968). All larvae exposed to concentrations of 0.8 ppm or greater died after 3 days exposure and those that survived concentrations of less than 0.8 ppm exhibited a reduced rate of shell growth compared to controls. Armstrong and Milliemann (1974b) exposed two larval stages (first polar body and 32 hour stage) of the bay mussel to carbaryl for 1 hour and then observed subsequent development to the 48-hour stage. The EC_{50}'s for anomalous development among larvae exposed to carbaryl as first polar body and 32 hour stages were reported to be 5.3 and 24.0 ppm, respectively. An EC_{50} for 1-naphthol for the first polar body stage, however, was reported to be 5.2 ppm. Roberts (1975) reported an EC_{50} greater than 30 ppm carbaryl for inhibition of attachment of byssal threads in the common mussel at both 24 and 48 hours of exposure. Growth of young oysters, however, was inhibited by 24 hours of exposure to 1 ppm carbaryl (Butler et al. 1962, in Roberts 1975).

Stewart et al. (1967) reported results for short-term exposures to Sevin. These authors indicated LC_{50}'s for carbaryl of 2.3 ppm for bay muscle larvae over 48 hours; 2.2 ppm for Pacific oyster larvae (24 hours), and 7.3 ppm for adult cockle clams (24 hours).

Carbaryl formulations are widely applied in agricultural situations. There is a high likelihood for earthworms (Oligochaete annelids) to be exposed to both carbaryl and its residues in the soil, with potential loss of their beneficial effects as soil conditioners. The toxic effects of carbaryl have been studied by several authors in a number of species of earthworms and in one aquatic oligochaete worm species. In general, the effects of carbaryl among earthworms were found to vary widely, with a high degree of species specificity evident in terms of lethal toxicity. For the most part, studies with carbaryl indicate that it is toxic to slightly toxic with most lethal effects reported in worms at concentrations above 1 ppm and in some species, above 10 ppm. Stenersen (1979a and 1979b) compared the toxic response of four species of earthworm to carbaryl in terms of LC_{50}'s following a 30-minute dip in a carbaryl solution and mortalities following 30 days exposure in soil mixed with carbaryl. A wide range of responses was evident in these two studies, with LC_{50}'s ranging from 3.1 to 6.3 ppm in one species to 200 to 800 ppm in another species. Exposure for 30 days in contaminated soils resulted in similarly variable results; a carbaryl concentration in soil of 4 ppm in soil was lethal to all worms of two species within 2 days, and no mortality was observed after 14 days of exposure to up to 64 ppm in soil in another species. In the species with the greatest tolerance for carbaryl (Eisenia foetida), sublethal effects were reported, including tonic tremors, inhibition of burrowing, and a decrease in cholinesterase activity. In another study with Eisenia foetida, Roberts and Dorough (1984) reported an LC_{50} for carbaryl of 9.0 μg/square centimeters (cm^2) of filter paper for 48 hours exposure. These authors considered carbaryl to be "extremely toxic" to earthworms. In the same study, Roberts and Dorough (1984) reported an LC_{50} of 0.28 $\mu g/cm^2$ for the species Lumbricus rubellus. Thompson (1971) reported that in Lumbricus terrestris, numbers of individuals were reduced to 40.2 percent of controls and that biomass was reduced to 31.7 percent of control levels following 3 weeks of exposure in field plots of soil following application of a wettable powder formulation of carbaryl at a rate of 2 lb a.i./acre (2.24 kg/ha). Kale and Krishnamoorthy (1982) reported that exposure in soil cultures to carbaryl for 1 month among populations of the worm Pontoscolex corethrurus resulted in an increase in acetylcholinesterase activity at concentrations below 100 ppm and a decrease in such activity at concentrations above 250 ppm. Naqvi (1973) exposed the aquatic tubificid worm (Branchiura sowerbyi) to carbaryl for as long as 90 days at different temperatures. A concentration of 4 ppm carbaryl resulted in 100 percent mortality within 72 hours at temperatures of 4.4 °C and at 32.2 °C. No mortality was observed at a temperature of 21 °C, however.

The toxic effects of carbaryl have been studied in two species of the predatory mite, Amblyseius (Euseius). Predatory mites are the natural predators of target species of carbaryl application and are thus potentially exposed, both orally and topically, to the insecticide during commercial applications. Hislop and Prokopy (1981) reported mortality rates of 100 and 89 percent, respectively, in two strains of adult females that had been dipped for 5 seconds in a solution of 119 g carbaryl/100 l and then observed for 48 hours. Among mites exposed to treated leaves, 48 hour mortalities of 38.4 to 90.3 percent were reported among larvae exposed to leaves treated with 450 to 1,800 mg a.i./l (milligrams of active ingredient per liter) concentration in one strain and 100 percent for larvae exposed to leaves treated with 225 to 900 mg a.i./l in another strain (Tanigoshi and Congdon 1983). Jeppson et al. (1975) reported that treatment of lemon leaves with carbaryl required a concentration greater than 10 ppm to prevent egg hatch, although a concentration of 10 ppm resulted in residues that prevented maturation of mites.

Carbaryl has been reported to be moderately to highly toxic in a wide variety of aquatic crustacea. For the most part, LC_{50}'s have been reported at concentrations less than 1 ppm. In some cases, LC_{50}'s between 1 and 10 ppm have been reported. Among the decapod crustaceans (which include crayfish, crabs, shrimp, and lobsters), several species of crayfish, shrimp, and crabs have been tested. LC_{50}'s for species of the crayfish, Procambarus, have been reported by Cheah et al. (1980a), Carter and Graves (1973, in Cheah et al. 1978 and 1980b), and Chang and Lange (1967). All of these studies indicate that carbaryl is highly toxic to Procambarus, with toxic effects seen at concentrations of less than 1 ppm. However, Chaiyarach et al. (1975) reported TL_{50}'s for Procambarus simulans of 2.87 to 2.43 ppm for 24 and 96 hours exposure, respectively, and Johnson and Finley reported a 96-hour LC_{50} of 1.90 ppm for an unspecified species of *Procambarus*, indicating a greater tolerance to carbaryl in these studies than was indiced by studies described above.

Butler (1964) reported field toxicity studies from hemlock looper control to aquatic crustacea in commercial fisheries in Washington. He reported impacts on Dungeness crabs, little-neck clams, and Pacific oysters, and found no detectable residues of carbaryl in these species with no direct evidence of crustacean mortality. Henrick et al. (1966) reported results of field tests from treatment of rice. The carbaryl applied at a rate of 1.0 lb a.i./acre (1.12 kg/ha) did not adversely affect the survival, growth, or reproduction of Louisiana red crawfish during the 8-month post-spray observation period. All studies with shrimp indicate that carbaryl formulations are highly toxic to this group of crustaceans. LC_{50}'s, TL_{50}'s, and EC_{50}'s for carbaryl of less than 1 ppm have been reported in studies with several species of shrimp (Chaiyarach et al. 1975, Hansen et al. 1973, Johnson and Finley 1980, and Nimmo et al. 1981). Hansen et al. (1973) reported that shrimp, when exposed to concentrations of 0.1 to 0.0001 ppm, did not show any tendency to avoid carbaryl in a Y-chamber during a 1-hour observation period.

Several studies have shown lethal and nonlethal effects of low concentrations of carbaryl in two species of crabs. Weis and Mantel (1976) reported that a carbaryl concentration of 0.1 ppm (length of time unspecified) was lethal to fiddler crabs. Buchanon et al. (1970, in Hart and Fuller 1979) reported that carbaryl concentrations of 1 ppm did not affect hatching of the Dungeness crab, but that molting was inhibited at 1 ppm and delayed at concentrations as low a 0.1 ppb. In the cladoceran (water flea), *Daphnia magna*, Lejczak reported LC_{50}'s of 22.90 to 3.28 ppm for 24 to 96 hours exposure. Other studies with several cladoceran genera (Johnson and Finley 1980 and Santharam et al. 1976) have all reported EC_{50}'s and TL_{50}'s of much less than 1 ppm, indicating that carbaryl is highly toxic to these organisms. In a stressed bioassay with *Daphnia*, Bowman et al. (1981 and 1982) observed 18-hour LD_{50}'s of 0.00505 g/individual and 0.008 g/individual when stressed with 0.4 percent and 2.0 percent methanol, respectively.

Carbaryl is very highly toxic to the scud genus *Gammarus*, with 96-hour LC_{50}'s of 0.022 to 0.026 ppm (Johnson and Finley 1980). Based on studies with another scud species, *Hyalella azteca*, Bowman et al. (1981 and 1982) reported stressed 18-hour LD_{50}'s of 0.0911 μg/individual and 0.016 μg/individual when stressed with 0.5 percent and 2.0 percent methanol, respectively. In other studies with crustacea, Johnson and Finley (1980) reported a 96-hour LC_{50} of 0.28-ppm carbaryl for the sowbug, (*Ascellus brevicaudus*, and Hansen and Kawatski (1976) reported 24 and 72 hour LC_{50}'s of 5.28 and 1.80 ppm carbaryl, respectively, for the ostracod, Cypretta kawatai.

The toxic effects of carbaryl and many of its commercial formulations have been reported in a wide range of studies with nontarget insect species. These studies are representative of a wide range of insect taxa. Of particular concern have been the potential toxic effects to bees following commercial agricultural and forest application of carbaryl, as well as the effects of carbaryl on insects that are normal predators of target species.

Studies on the effects of carbaryl have shown it to be toxic to bees both by the oral route and by contact. Argauer et al. (1972) reported 50 percent mortality among bees 24 hours after ingestion of 0.2 μg carbaryl/bee in a sugar solution, Johnson and Brown (1972) reported up to 39 percent mortality among bees that had ingested pollen contaminated with carbaryl, Moffett et al. (1970) reported that pollen contaminated with carbaryl to achieve an initial concentration of 100 ppm retained residual toxicity to bees after 2 months of weathering, Redfern and Knox (1974) reported 70 to 100 percent mortalities 48 hours after bees had ingested 0.25 to 1.0 μg carbaryl/bee, and Stevenson et al. (1977) reported an acute LD_{50} of 0.14 μg/bee. Topical application of carbaryl to bees has been reported in studies by Atkins et al. (1981), Bai and Reddy (1977a and 1977b), Barker (1970), Graves and Mackensen (1965), Ibrahim et al. (1971), Redfern and Knox (1974), Singh et al. (1974), and Stevenson et al. (1977) These studies have indicated toxic effects in both laboratory and field. For the most part, the studies have been conducted at rates of application or concentrations equivalent to those found under normal commercial agricultural use. In other studies with bees, Anderson and Glowa (1984) reported carbaryl in 28 of 43 samples of bees following agricultural application of carbaryl, Johansen (1972) reported mortalities of 89 to 100 percent among four species of bees placed on leaves that had been treated with carbaryl at a rate equivalent to 1 lb a.i./acre (1.12 kg/ha) and then weathered for 2 days, and Keener and Pless (1974) reported moderate toxicity (15 percent mortality within 6 hours) among bees exposed to carbaryl applied as a 1 percent suspension to saw cuts on the hive.

Research at the University of California (Atkins 1981) has shown that some formulations of carbaryl are 2 to 4.5-fold safer to honey bees in field applications. Slight alterations of the physical properties of each formulation significantly reduces the hazard to honeybees to acceptable levels under certain circumstances. The variety of formulations tested in the field resulted in a range in bee hazard of extremely high (2750 to 4000 bees/colony killed) to moderately low (150 to 350 bees/colony killed). The LD_{50}'s ranged from 1.5 to 26.5 μg/bee. The latter was due to resistance to wash-off of the carbaryl in rain and irrigation.

Carbaryl was reported to cause reduced emergence among larvae of the silkworm, another important commercial insect (Kuribayashi 1981). The survivors of larvae that had been fed leaves treated with 0.004 and 0.008 percent carbaryl laid fewer eggs, more of which were abnormal and had a lower hatch success for their eggs, compared to controls.

Olson-Elliott (1976) reported field results in Montana following use of carbaryl and trichlorfon to control spruce budworms. No significant difference in bee densities was observed between the spray and control sites. No consistent reduction in small bees (Megachilidae, Adrenidae, Halictidae, and Colletidae families) was observed following the carbaryl treatment. Except for snowberry, there were no consistent reductions in fruit production linked to pollination. Holmes at al. (1981) reported results of field studies in Canada, following the application of Sevin-2-Oil to control spruce budworm. Carbaryl was applied twice at a dose rate of 280 g/ha/application (grams per hectare per application). No significant adverse effects were reported for either songbirds or wild pollinators, while a "slight knockdown effect" was reported for nontarget arthropods. No adverse effects on trout and negligible effects on the benthic aquatic invertebrates of streams were observed.

In other studies with insects, toxic effects have been reported for carbaryl at relatively low concentrations for most organisms. In the mosquito species Aedes aegypti, a concentration of 1 ppm carbaryl resulted in a 24-hour larval mortality of 70 percent (Lichtenstein et al. 1966). The 18-hour LD_{50} for the mosquito Culex restuans in a stressed bioassay with 0.5 percent methanol was reported to be 11.1 μg/organism (Bowman et al. 1981 and 1982). Among aquatic stoneflies, a sensitive indicator, technical carbaryl (99.5 percent) was highly toxic to all of four stonefly genera tested and an oil dispersion formulation (49 percent carbaryl) was highly toxic to the one genus tested, with 96-hour LC_{50} of much less than 1 ppm (0.0017 to 0.0056 ppm for technical material and 0.0092 ppm for oil dispersion material) (Johnson and Finley 1980). Carbaryl was moderately toxic to the midge, with a reported 72-hour LC_{50} of 5.90 ppm (Hansen and Kawatski 1976).

In studies with a variety of aquatic insects or aquatic stages of terrestrial insects, carbaryl has been reported to have lethal effects at concentrations below, and in some instances well below, 1 ppm (Bradt and Williams 1980, Federle and Collins 1976, Jeyasingam et al. 1978, Shukla and Mishra 1980, Shukla et al. 1982). In the crawling water beetle (Peltodytes sp.), Federle and Collins (1976) reported LC_{50}'s ranging from 100 ppm for 24 hours of exposure to 3.3 ppm for 96 hours of exposure.

McCullough and Stanley (1980) reported the results to stream ecosystems following field applications of carbaryl in Maine. While the pesticide reached the control stream, no major effects on the aquatic community were detected. Stonefly nymphs (Plecoptera), usually a sensitive species, were not affected by carbaryl residues, nor were other invertebrates. The abundance of benthos was apparently not affected by carbaryl. In previous studies, however, carbaryl significantly altered the composition of benthos in Maine streams. The lower standing crop measured later in the season was probably not a result of a delayed action of the carbamate (McCullough and Stanley 1980).

Among the lacewings, Brettell (1979) reported a 24-hour LC_{50} equivalent to 4.39 percent a.i. and Plapp and Bull (1978) reported an LC_{50} of 104 µg/vial for surface absorption among larval green lacewings. Reeve and French (1978) reported 90 percent mortalities both 24 and 48 hours after application of a concentration equivalent to 5.6 lb a.i./250 gal to brown lacewings. Jordan et al. 1979 reported 100 percent mortality of cricket eggs (Acheta domesticus) after application of an 0.1 percent solution; some survivors of 0.01 percent concentrations showing abnormal limb development. There were no differences compared to controls in hatching success at concentrations of 0.0005 percent or less. Topical application of 0.1 µg carbaryl/bug to the large milkweed bug caused 40 percent mortality within 24 hours, application of 5.0 µg/bug caused 100 percent mortality within 24 hours (Argauer et al. 1972). Among individuals of the western big-eyed bug exposed to residues of carbaryl, Yokoyama et al. (1984) reported 24-hour LD_{50}'s equivalent to an application rate of 1.6 lb/acre (1,790.5 g/ha) and 3.5 lb/acre (3,923.8 g/ha), respectively, on leaves and on filter paper. On treated soils, an application rate equivalent to 1 lb a.i./acre (1.12 kg/ha) resulted in a mortality of 38 percent within 2 days among striped earwigs (Workman 1977). One milliliter of an 0.1 percent solution, applied to 20 larvae in a petri dish, resulted in 66.66 percent mortality within 12 hours and 97.61 percent mortality within 72 hours (Makar and Jadhav 1981). Among predatory beetles of the genus Sterthorus, Edwards and Hodgson (1973) reported mortalities of 100 percent among insects that had been dipped in an 0.11 percent solution for 15 seconds and Walters (1976) reported that exposure to filter paper, treated with an 0.1 percent solution of carbaryl, resulted in 100 percent mortality among adults within 48 hours and 100 percent mortality among larvae within 48 hours among three species tested. For another predatory insect, Reduviolus americoferus, Martinez and Pienkowski (1983) reported a 24-hour LC_{50} of 173.91 ppm for a 50 percent wettable powder formulation of carbaryl following immersion for 2 seconds in solution.

2.3 Fish

Numerous studies have addressed the potential adverse effects of carbaryl and its formulations to fish. These studies have provided information about the sublethal effects of carbaryl as well as the lethal toxicities of carbaryl and several of its formulations. For the most part, the studies summarized below and described in detail in appendix C, indicate that 96-hour LC_{50}'s (or the equivalent, TL_{50}'s) range from 1 to 10 ppm carbaryl, although some studies have reported LC_{50}'s greater than 10 ppm and some studies have indicated LC_{50}'s of less than 1 ppm. Clarke et al. (1970) proposed that chemicals with fish LC_{50}'s of less than 1 ppm be considered highly toxic, that chemicals with fish LC_{50}'s of greater than 10 ppm be considered slightly toxic, and that chemicals with fish LC_{50}'s of 1 to 10 ppm be considered toxic. For the most part, carbaryl formulations should be considered moderately toxic to most fish species.

Several studies described in appendix C are comparative and have addressed the tolerance of different fish species to carbaryl, or they have addressed the varying effects of temperature, other environmental variables, or differing formulations on the concentrations of carbaryl that fish can tolerate. Bansal et al. (1980) exposed the larvae of four species of freshwater carp to a wettable powder formulation of carbaryl. They reported TL_{50}'s ranging from 1.73 to 2.00 ppm and estimated 30-day maximum acceptable toxicant concentrations ranging from 0.052 to 0.078 ppm. Johnson and Finley (1980) compared 96-hour LC_{50}'s for nine species of fish exposed to technical carbaryl (99.5 percent material) in a static bioassay, for one species exposed to technical carbaryl in a flow-through bioassay, and for two species exposed to a 49 percent oil dispersion formulation in a static bioassay. Of the 12 LC_{50}'s reported in this study, the LC_{50} for bluegill exposed to oil dispersion formula in a static test was 39.0 ppm and the LC_{50} for lake trout exposed to technical carbaryl in a static test was 0.69 ppm. The remaining LC_{50}'s in the study ranged from 1.95 to 7.10 ppm. Johnson and Finley (1980) observed that a temperature increase from 7 °C to 17 °C resulted in a small alteration in the LC_{50} of carbaryl to cutthroat trout. A similar increase in temperature, however, resulted in a 4- to 11-fold increase in the toxicity of carbaryl to brook trout and to yellow perch. Under alkaline conditions (pH 8.5 to 9.0), test solutions were 1.4 to 11.4 times more toxic to trout, salmon, and to yellow perch than were slightly acidic to neutral solutions (pH 6.5 to 7.5). In another comparative study with twelve species of fish exposed to technical material (99 percent) in static bioassay tests, Macek and McAllister (1970) reported LC_{50}'s of 11.2 to 20.0 ppm for five species, of 1.95 to 6.76 ppm for five species, and 0.745 to 0.764 ppm for two species. In this study, the most tolerant fish were catfish (Ictalurids) and the least tolerant were salmon, trout, and perch (Salmonids and Percids). Post and Schroeder (1971) reported TL_{50}'s for four species of salmonid fish (brook trout, cutthroat trout, rainbow trout, and coho salmon) exposed to 98 percent carbaryl under static bioassay conditions. The 96-hour TL_{50}'s ranged from 1.070 to 2.169 ppm, with the larger brook trout and cutthroat trout tending to have a greater tolerance to carbaryl than did smaller fish. Sanders et al. (1983) reported 96-hour LC_{50}'s for four species of fish exposed under static bioassay conditions to technical carbaryl (99.5 percent material). The most sensitive species was the rainbow trout, with an LC_{50} of 2.2 ppm. The remaining three species, bluegill, fathead minnow, and channel catfish, had

LC_{50}'s ranging from 7.0 to 15.8 ppm carbaryl. Schoettger and Mauck (1978) reported 96-hour LC_{50}'s of 0.9 to 5.4 ppm for brook trout exposed to technical carbaryl (99.5 percent material) under static conditions at different temperatures, hardness, and pH. They reported that the toxicity of carbaryl to brook trout increased with an increase in temperature, with an increase in hardness, and with an increase in pH. Tilak et al. (1979 and 1981) reported the 96-hour LC_{50}'s for five species of freshwater fish exposed to carbaryl and to the primary metabolite of carbaryl, 1-naphthol, in a flow-through bioassay test. The LC_{50}'s for carbaryl ranged from 2.4 to 6.4 ppm and for 1-naphthol ranged from 0.33 to 4.3 ppm. In a comparative study of the toxicity of different formulations of carbaryl at differing temperatures, pH, and softness, Woodward and Mauck (1980) reported a range of LC_{50}'s ranging from 0.97 to 6.0 ppm for technical material and of 6.7 ppm (a.i.) for the 49 percent formulation. They further reported that the trout were more sensitive to carbaryl as the temperature was increased and as the pH was increased.

Most of the 96-hour LC_{50} values reported in the literature for fish were greater than 1 ppm, although several authors have reported LC_{50}'s of less than 1 ppm for a shorter duration than 96 hours. Arunachalam et al. (1980) reported a 96-hour LC_{50} of 3.5 ppm in the air-breathing fish Macropodus cupanus in a static bioassay with a 50 percent wettable powder formulation of carbaryl. One hundred percent of the fish survived at a concentration of 2.5 ppm, and 100 percent died at a concentration of 4.5 ppm. Sublethal concentrations (up to 2.5 ppm) resulted in increased rates of surfacing, swimming, and metabolism, and in a decreased growth rate and food conversion efficiency compared to control fish. Bashra et al. (1983) reported a 48-hour LC_{50} of 5.495 ppm in a static bioassay with the freshwater fish Tilapia mossambica. Bhatia (1971) reported a static 96-hour TL_{50} of 3.7 ppm in the fish Puntius ticto exposed to 99 percent technical carbaryl. Based on a static bioassay with channel catfish exposed to 100 percent pure carbaryl, Brown et al. (1979) reported a 96-hour median toxic limit of 1.30 ppm. Chin and Sudderuddin (1979) reported a 96-hour LC_{50} of 1.7 ppm in a static test with common carp exposed to 94 percent a.i. material. In the tilapid fish, Sarotherodon mossambica, a 72-hour LC_{50} of 8 ppm was reported for a 50 percent wettable powder formulation of carbaryl (Koundinya and Ramamurthi 1980b). Li and Chen (1981) reported 48-hour TL_{50}'s in a tilapid and in the mosquito fish, respectively when exposed to technical (99 percent) carbaryl. In an estuarine fish (Therapon jarbua) exposed in a static test to carbaryl, the 96-hour LC_{50} was reported by Lingaraja and Venugopalan (1978) to be 2.20 ppm. The authors further observed that exposure of this species to 0.62 ppm (the LC_{25}) resulted in elevated respiratory rates compared to controls. Pant and Singh (1983) reported a 96-hour static LC_{50} of 2.142 ppm for a 50 percent wettable powder formulation in the fresh water cyprinnid fish Puntius conchonius. They also observed that the exposed fish had significant hyperglycemia and in some organs, glycogenolysis.

Long-term exposure to concentrations of 0.194 and 0.306 ppm for 15 to 30 days resulted in hypoglycemia and in depletion of liver glycogen. Van Hoof (1980) reported death within 4.5 to 5.5 hours among rainbow trout exposed to 1.5 ppm carbaryl. Verma et al. (1981 and 1984) reported a static 96-hour TL_{50} in the carp species, Cyprinus carpio, of 2 ppm for carbaryl, and a static 96-hour LC_{50} in the carp Cirrhina mrigula of 1.94 ppm for a 50 percent wettable powder formulation. In the latter study, an estimated maximum allowable toxicant concentration of 0.0873 to 0.1086 ppm was calculated based on 60 days of exposure to sublethal concentrations of carbaryl.

Several studies have indicated toxicities in fish that suggest slight toxicity of carbaryl, with LC_{50}'s of approximately 10 ppm or more. Arunachalam et al. (1980) reported a 72-hour LC_{50} of 17.5 ppm in catfish exposed to a 50 percent wettable powder formulation under static test conditions. Mortalities were 100 percent within 24 hours at a concentration of 32.5 ppm, and survival was 100 percent after 72 hours at a concentration of 12.5 ppm. In the mosquito fish, the 96-hour TL_{50} for an 80 percent wettable powder formulation under static test conditions was 31.80 ppm (Chaiyarach et al. 1975). Lejczak (1977) reported an LC_{50} of 9.74 ppm for the fish Libistes reticulatus in a static bioassay, but noted that distinct pathology was observed at concentrations as low as 6.2 ppm. Singh et al (1984) reported 96-hour TL_{50}'s for static conditions with a 50 percent wettable powder of 19.50 and 20.10 ppm for two freshwater teleost fish. Exposed fish displayed restlessness, erratic opercular movements, convulsions, and they dove to the bottom of the tank where they ultimately died. Toor and Kaur (1974) reported a 72-hour LC_{50} of 10.36 ppm in carp exposed under static conditions to a 50 percent wettable powder formulation of carbaryl. They also reported that at a concentration of 13.5 ppm, exposed fish were initially excited, then swam slowly on their side, exhibited a decreased rate of respiration, and eventually died at the bottom of the tank. The 96-hour LC_{50} for a 50 percent wettable powder formulation in a cyprinnid fish under static conditions was reported by Verma et al. (1982) to be 19.58 ppm.

In addition to these studies, many authors have been primarily concerned with nonlethal and/or long-term toxic effects of carbaryl and its formulations on fish species. These latter studies have been concerned with effects on AChE activity, reproductive function, hematology, and behavioral changes.

Several studies have addressed the effects of carbaryl on AChE activity. Coppage (1977) exposed pinfish to carbaryl in a flow-through system and reported a significant ($p < 0.005$) reduction in acetyl cholinesterase activity to 79 percent of that found in brain tissues of controls among fish that had been exposed for 15 hours to a 1.3-ppm concentration of carbaryl. The concentrations of carbaryl used in this study resulted in 40- to 60 percent mortality of the test fish in this study. Lowe (1967) reported a 17 percent inhibition in brain AChE activity after 2.5 months of exposure and normal activity after 5 months of exposure to 0.1 ppm carbaryl in a flow-through system. Exposure to 1.0 ppm for 13 days resulted in a 32 percent inhibition of AChE activity in this study. Koundinya and Ramamurthi (1979b) reported that exposure to the lethal concentration of 10 ppm carbaryl among the tilapid fish Sarotherodon mossambica resulted in a significant ($p < 0.001$) decrease in AChE activity and consequent increase in acetylcholine content in various tissues. In a field study where Sevin-4-oil had been applied twice at 0.5 lb/acre (0.56 kg/ha) with a 7-day interval between applications, brain AChE activity among brook trout that were sampled from local streams was significantly ($p = 0.05$) lower in exposed fish than in unexposed fish 3 days after the first application and the day of the second application (Haines 1981). This difference disappeared within 24 to 48 hours, however.

McCullough and Stanley (1980) and Ott et al. (1981) reported field results following carbaryl use in Maine to control spruce budworm. Neither study recorded adverse affects on brook trout or their food supplies. While the carbaryl drifted after aerial application, it did not adversely impact streams. Ott et al. (1981) found residues for 2 days following application; however, maximum residue was below toxicity levels for brook trout. A sensitive index to pesticide exposure, the ChE enzyme, was not altered in the brook trout fry. Similar results were reported by McCullough and Stanley (1980), who found no differences in ChE and no young trout affected following the spray program. Earlier in Maine, Hulbert (1977) found short-term (48 hour) ChE depressions in brook trout and landlocked Atlantic salmon following applications of carbaryl at rates of 0.75 lb a.i./acre (0.84 kg/ha). Post-spray residues quickly dissipated. Similar short-term but insignificant effects were reported by Tompkins (1966). He found a 0.017 ppm increase in 1-naphthol content (a carbaryl metabolite) as a residue in 479 fish, following spraying of carbaryl at a rate of 1 lb of a.i./acre (1.12 kg/ha). Following exposure to high carbaryl levels in water, fish eliminated 91 percent after 2 hours and 100 percent after 24 hours.

Long-term reproductive studies have, in several cases, indicated that carbaryl can adversely affect the development and reproductive function of fish. Carlson (1971) exposed fathead minnows to concentrations of up to 0.68 ppm carbaryl for 9 months in a flow-through system. At the highest concentration, survival was decreased and reproduction was inhibited due to retention of sperm in the males and resorptive ovaries in the females. The authors also reported a 96-hour TL_{50} of 9.0 ppm and minimum acceptable toxicant concentration of 0.21 to 0.68 ppm based on long-term exposure. Kaur and Toor (1977) exposed carp eggs to carbaryl through the hatching stage and reported an LC_{50} of 1.4 ppm for hatching. Exposed fish exhibited deformities such as enlarged pericardial sacs, circulatory failure, and curling of the posterior region of the body. Sublethal concentrations of carbaryl (10 and 20 ppm) in a fresh water teleost (Channa striatus) adversely affected reproductive systems by causing a decreased percentage of maturing and mature oocytes, rupture of oocyte walls, and dilation of blood vessels in fish exposed for 30 days (Kulshrestha and Arora 1984). Solomon (1977) and Solomon and Weis (1979) reported a variety of circulatory abnormalities in developing medaka. More than 80 percent of eggs exposed under static conditions to 5 ppm carbaryl from fertilization through hatching exhibited anomalies such as rudimentary heart development, pericardial edema, irregular heartbeat, backflow of blood and clots in the atria, and extra-embryonic circulation. Weis and Weis (1974a) reported developmental arrest prior to the initiation of heartbeat among killifish exposed as early embryos to 10 ppm carbaryl.

Several studies in fish have reported various physiological effects of sublethal and lethal concentrations of carbaryl to fish. Koundinya and Ramamurthi (1979a and 1980a) reported significant decreases in red blood cell count, packed cell volume, and hemoglobin concentration among tilapid fish (Sarotherodon mossambica) exposed to a lethal (10 ppm) concentration of carbaryl as well as to a sublethal (4 ppm) concentration. Sastry and Siddiqui (1982) exposed the freshwater teleost Channa punctatus to 1.05 ppm carbaryl for 60 days and reported a pattern of altered enzyme, glycogen, and lactic acid levels that were indicative of a favored anaerobic metabolism and impaired aerobic metabolism among exposed fish. Killifish exposed for 2 weeks to 0.01 ppm carbaryl exhibited reduced regeneration of the caudal fin (Weis and Weis 1975).

Relatively low concentrations of carbaryl have been shown in several studies to result in the disruption of behavior among exposed fish. Hansen (1969) reported that sheepshead minnows, exposed for 1 hour in a Y-maze, did not show an avoidance to carbaryl at concentrations of 10, 1.0, or 0.1 ppm. Mosquitofish in a Y-maze exhibited a preference for freshwater when given the choice between freshwater and 10 ppm carbaryl. However, there was no preference for freshwater over either 1.0 ppm or 0.1 ppm carbaryl, nor did the fish appear to be able to discriminate between 10 ppm and 1.0 ppm carbaryl (Hansen et al. 1972). Peterson (1976) tested the effect of carbaryl concentrations of 0.25 to 1.0 ppm on the temperature preference of juvenile Atlantic salmon in a temperature gradient and reported that carbaryl concentrations of up to 1.0 ppm did not alter the temperature preference of the test fish. Weis and Weis (1974b) reported that a single application of 0.100 ppm carbaryl disrupted the schooling behavior of silversides (Menidia menidia) within 24 hours of exposure and postulated that this disruption may have been due to the accumulation of the toxic metabolite, 1-naphthol.

Several studies have evaluated the effects of carbaryl in ranid frogs and in the African clawed frog. Tucker and Crabtree (1970) reported an acute oral LD_{50} of 4,000 mg/kg for the bullfrog (Rana catesbieana) administered a single dose of a 50 percent wettable powder formulation by capsule and observed for as long as 14 days. In the African clawed frog (Xenopus laevis), an LC_{50} of 4.7 ppm was reported for surface absorption in embryos (Elliott-Feeley and Armstrong 1982). In this study, an EC_{50} for abnormalities leading to post hatching death was 0.11 ppm. Embryos that were treated with 10 ppm carbaryl prior to gastrulation exhibited internal abnormalities such reduced or absent heart, occlusions of the notochord or of the spinal cord, lysis of yolk platelets, and edema. Rzehak et al. (1977) reported effects of carbaryl (Karbatox 75) to European frogs (Rana temporaria) and to the African clawed frog (Xenopus laevis) with as long as 8 and 10 days maximum exposures, respectively. In the European frog, exposure to 0.1 percent carbaryl resulted in 100 percent mortality within 10 to 12 hours, whereas exposure to 0.001 percent carbaryl resulted in 90 percent mortality within 7 to 10 days. The survivors of the 0.001 percent carbaryl exposure died within 2 days after transfer to fresh water. In the clawed frog, exposure to 0.01 percent carbaryl for 24 hours resulted in 80 percent mortality within one week after return to fresh water, and 100 percent mortality within three weeks after return to fresh water, while exposure to 0.001 percent for 8 days resulted in 20 percent mortality one week after return to fresh water and 65 percent mortality two weeks after return to fresh water. In both species, Rzehak et al. (1977) reported that exposed animals exhibited hyperactivity followed by slow swimming and sinking to the bottom of the tank.

2.4 Birds

A number of studies are available on acute and long-term effects of carbaryl on birds. These studies are summarized below and in table 2-1 and are described in appendix D.

Studies on the acute oral toxicity of carbaryl to birds indicate that formulations have a slight to moderate toxicity to most species. El-Dakoury et al. (1979a) dosed hens orally with a single dose 0, 100, 150, 250, 350, or 450 mg/kg body weight by capsule and observed behavior and survival for 24 hours. They reported an LD_{50} of 248 mg/kg. A dose of 450 mg/kg resulted in 100 percent mortality. Schafer (1972) reported an LD_{50} of 56 mg/kg for acute exposure to carbaryl administered by gavage to redwing blackbirds. Solomon and Robel (1980) administered a single dose of 10, 50, or 90 mg carbaryl/kg body weight into the crop of bobwhites. They reported no significant changes in body weight, energy intake, energy excretion, energy metabolized, or metabolic efficiency in exposed birds compared to controls during and 8-day observation period. No deaths were reported among treated animals. Tucker and Crabtree (1970) administered a single oral dose by capsule of several formulations to mallards, pheasants, coturnix quail, pigeons, sharp-tailed grouse, and Canada geese. They reported acute oral LD_{50}'s ranging from a low of 707 mg/kg body weight in pheasants to a high of 3,000 mg/kg body weight in pigeons. Haegle and Turner (1974) reported that a single oral dose of carbaryl (1,000 mg/kg body weight), administered by capsule to coturnix quail and mallards caused no appreciable thinning of the egg shell, with less than a 5 μm reduction in thickness between eggs laid before and after administration of the carbaryl dose.

There were no overt signs of toxicity among Japanese quail dusted with a 5 percent carbaryl formulation at estimated exposures of 140 to 160 mg/kg and observed for 24 hours (Hill 1979). A slight but significant ($p < 0.05$) reduction in plasma ChE was observed 6 hours after treatment but levels returned to normal by 24 hours after treatment. No changes were observed in brain ChE, however.

Gaines (1968) reported neurotoxic effects within 24 hours and lasting for 24 days among chickens given an acute subcutaneous injection of carbaryl after having first been injected with 15 mg atropine/kg body weight to protect against acute poisoning. Effects reported included leg weakness and paralysis.

Several studies have addressed the potential subchronic oral toxicities of carbaryl formulations. Bursian and Edens (1977) fed Japanese quail a diet containing 0, 50, 150, 300, 600, 900, or 1,200 ppm carbaryl from hatching through 14 weeks of age. Some effects on body weights and on relative organ weights were noted at higher concentrations. Animals of both sexes on a diet containing 150 ppm carbaryl or more exhibited increased kidney weights. Males fed 900 and 1,200 ppm exhibited decreased body weights and females fed 900 ppm carbaryl in the diet had decreased body weights. No significant effects were noted on egg production or hatchability, although there was a slight decrease in egg production among individuals fed a diet containing 600 ppm or more carbaryl. No gross teratogenic effects were observed. There were significantly elevated whole brain dopamine levels among males fed 600 and 1,200 ppm carbaryl in the diet, but no effects were noted among females. No differences were noted in levels of whole brain AChE activity, whole brain and heart norepinephrine levels, adrenal norepinephrine and epinephrine levels, or plasma glucose levels. Hill et al. (1975) reported no mortalities due to carbaryl in the diet for 5 days among bobwhites fed 2,400 ppm or Japanese quail, ringnecked pheasant, or mallards fed 5,000 ppm in the diet. Nir et al. (1966) fed Opigal (kaolin powder with 50% Sevin) by capsule to White Leghorn hens daily for 60 days at a rate equivalent to 180 or 540 mg Sevin/kg body weight. They reported no mortalities after 60 days among hens dosed at 180 mg/kg/day and 100 percent mortality within 35 days among hens dosed at 540 mg/kg/day. In the high-dose group, hens were restless after 3 days of dosing, had intensive diarrhea, decreased food consumption, liver degeneration, interstitial myocarditis, degenerated ovaries, atrophy of the spleen, and enlarged gall bladders. There were no changes through day 21 in blood chemistry or hematocrit, however. Robel et al. (1982) administered carbaryl in the diet of bobwhites for 7 days and reported no effects on weight of food consumed, weight of birds, or daily locomotor activity among birds fed 237 or levels 1,235 ppm carbaryl. Rodgers and Robel (1977) reported that ingestion of 4, 18, or 30 mg of carbaryl/kg/day for 15 days in the diet of adult male bobwhite did not alter fat reserves or body weight of underweight animals compared to controls. Zeakes et al. (1981) treated bobwhite with low doses of carbaryl orally by pipette for as long as 25 days and reported that as low as 10 μg of carbaryl increased the susceptability to infection by Histomonas meleagridis.

Studies by several authors have reported on the potential toxicity and teratogenicity of carbaryl formulations when injected into developing eggs of birds or when eggs were otherwise exposed to carbaryl. Hoffman and Albers (1984) dipped mallard eggs into a carbaryl solution for 30 seconds on the third day of incubation and examined living embryos for malformation on day 18 of incubation. They reported an LC_{50} greater than an equivalent of 216 lb/acre (242 kg/ha) in aqueous medium and greater than 24 lb/acre (26.9 kg/ha) in an oil carrier. No malformations were observed among eggs dipped in material in an aqueous carrier. In an oil carrier, there was reduced growth and there was an increase in the number of abnormal survivors compared to controls. These authors reported an LD_{50} of greater than 118 μg/egg in an oil carrier. Marliac (1964) reported that 1 mg carbaryl/egg resulted in malformations of chickens, but no details were provided. Proctor and Casida (1975) and Proctor et al. (1976) injected doses of 0.1, 0.3, 1.0, or 3.0 mg of carbaryl/egg into the yolk of hens eggs on day 4 or 5 of incubation. Body weight decreased and leg and body length decreased with increasing dose of carbaryl. There was an increase in the incidences of straight legs and of abnormal feathers as dose increased. Treated eggs had lower levels of nicotinamide adenine dinucleotide (NAD) than controls, indicating an association between NAD depression and teratogenesis. Chicken eggs injected with up to 10 mg carbaryl/egg showed significant increases in mortality after exposure for 5 and 12 days (Swartz 1981). Embryos exposed for 12 days exhibited fluid accumulation below the epidermis, cleft foot, tonically flexed digits, and unilateral ophthalmia. Tos-Luth et al. (1973) reported an LD_{50} of 3.44 mg/embryo following injection of carbaryl into the allantoic cavities of 10- to 11-day-old embryos. Varnagy (1981) injected Japanese quail eggs in the air space with 0.01, 0.10, or 1.0 mg/egg of an 85 percent wettable powder formulation of carbaryl on day 9 of incubation. Mean embryonic weights were higher than controls for the two lower doses and less than controls for the highest dose. One egg of 10 treated with 0.10 mg exhibited defective development of the skull and cervical vertebrae. One each of the 0.10 and 1.0 mg/egg groups died during the first 8 days and during days 9 to 10; no deaths were observed during days 11 to 14 when observation was terminated.

Two studies have addressed the effects of applications of carbaryl on birds. Moulding (1976) reported that application of 1 lb/acre (1.12 kg/ha) two times resulted in a significant decrease in field populations of birds in terms of numbers of birds, numbers of species, and species diversity, compared to controls during a 6-week observation period. Bahl and Pomeroy (1978) reported on mortalities of turkeys in a turkey house that had been treated with carbaryl at an application rate 14 times the recommended application rate. They reported 10-day mortalities of 16.4 percent, with tissue concentrations of 1.7 and 3.6 ppm in skin and fat, respectively.

A number of studies have shown minor adverse effects to wild bird populations, following the application of carbaryl in the field. In New York, Connor (1960) recorded effects on birds of applying Sevin at a rate of 1.25 lb a.i./acre (1.40 kg/ha) to control gypsy moths. His observations on 49 species of birds failed to show any effects on behavior, condition, reproduction, or rearing of young. No data were recorded on young birds lost as a result of spraying. Doane and Schaefer (1971) reported results in Connecticut following application of Sevin at a rate of 1 lb a.i./acre (1.12 kg/ha) for gypsy moths. Results suggest a difference (for Sevin plots, 42 percent decrease) in number of species seen after treatments. The authors believed that the aerial application had an indirect effect upon birds because it lead to reduced food biomass for birds. No mortality of nesting birds resulted from the spray. Holmes et al. (1981) evaluated the effects of carbaryl on forest songbirds. Two treatments of Sevin-2-Oil of 0.25 lb a.i./acre (0.28 kg/ha) applied 6 days apart had no significant adverse effect on songbirds. Breeding activities of high-risk species (least flycatcher, ruby-crowned kinglet, solitary vireo, black-throated green warbler, blackburnian warbler, and bay-brested warbler) were unaffected. DeWeese et al. (1979) monitored Montana forests after application of Sevin-4-Oil at a rate of 1 lb a.i./acre (1.12 kg/ha) for western spruce budworms. No significant differences in bird numbers in the field were reported following the spray. In the carbaryl plots, 92 percent of the breeding pairs remained. No sick or dead birds were found. The nesting success (i.e., survival of young), was 100 percent in carbaryl-treated plots, compared to 97 percent in control plots. Willcox (1972) monitored nesting success and weight of nestlings after applications of carbaryl at rates of 1 lb a.i./acre (1.12 kg/ha). Little, if any, weight loss occurred, and no insecticide-induced nestling mortality was noted.

Richmond et al. (1979) reported the impacts of carbaryl treatment at a rate twice that above (2 lb a.i./acre) (2.24 kg/ha) on birds in Oregon. No abnormal behavior, no ill birds, or any major changes in total numbers of breeding pairs were noted. Only 2 of 55 birds showed slight depression of ChE; however, neither was near the 50 percent lethal level. Zinkl et al.

(1977) measured the ChE activity in birds following carbaryl treatment in the field. Three birds of 90 had reduced ChE levels, 20 percent below prespray levels. Carbaryl had little effect on brain ChE activity in birds. Similar results were reported on ChE levels following carbaryl treatment in Maine (Gramlick 1979).

2.5 Mammals

The toxicity and potential health hazards from exposure to carbaryl have been evaluated in a variety of laboratory mammals, including rats, mice, rabbits, and dogs. Several reports have also addressed the effects of carbaryl on humans. The results of these various studies and toxicity tests are discussed in this section and are summarized in table 2-1 and described in appendices E, F, and G.

A metabolite of carbaryl, N-nitrosocarbaryl, has been studied for toxic effects in rats. Toxic effects of this metabolite are discussed in section 2.5.8 and are summarized in table 2-3 and described in appendices E-1 and G-1.

2.5.1 Acute Toxicity

Data from acute toxicity studies have been reported for oral, intraperitoneal, dermal, intravenous, eye irritation, and inhalation routes of exposure in several species of mammals.

2.5.1.1 Oral. Carbaryl and carbaryl formulations exhibit moderate to high acute toxicity to all mammals tested as indicated by the low LD_{50} values for acute oral studies. The oral LD_{50}'s for carbaryl in rats has been reported to be 500 mg/kg for females and 850 mg/kg for males (Gaines 1969 in Kuhr and Dorough 1976). The oral LD_{50}'s for carbaryl in mice (Rybakova 1966, in International Agency for Research on Cancer 1976) and rabbits (Carpenter et al. 1961) were reported to be 438 mg/kg and 710 mg/kg, respectively. According to U.S. Environmental Protection Agency toxicology guidelines, these toxicity ranges place carbaryl into Toxicity Categories II or III, warranting its use with warning and caution (Ashton 1982).

In Cranmer (1986) the author listed 13 studies of carbaryl and carbaryl mixtures used since 1979. For oral toxicity, the LD_{50} for technical carbaryl from one study (Bushy Run Research, in Cranmer 1986) on fasted rats reported the following acute oral results:

LD_{50} = 246 mg/kg (female)
LD_{50} = 283 mg/kg (male)

For 99 percent technical carbaryl, the LD_{50} in rats was 233 mg/kg.

Table 2-3

Summary of Toxicological Properties of N-nitrosocarbaryl

1. Carcinogenicity

Organism (Formulation)	Exposure	Adverse Effects
Rat	Single subcutaneous injection of N-nitrosocarbaryl at 1 g/kg.	Carcinogenic
Rat	Oral administration by gavage of N-nitrosocarbaryl twice a wk for 20 wks. Total dose was 300 mg.	Carcinogenic
Rat	Weekly oral administration by gavage of N-nitrosocarbaryl for 10 wks. Total dose was 50 mg.	Carcinogenic
Rat	Single oral administration by stomach tube of N-nitrosocarbaryl at 200 to 1,500 mg/kg.	Not carcinogenic

2. Mutagenicity

Organism (Formulation)	Exposure	Adverse Effects
Salmonella typhimurium, 5 test strains	0.00115, 0.0115, 0.115, 1.15, or 11.5 μg/plate.	Mutagenic
Saccharomyces cerevisiae	Test concs. were 0, 1, 5, 10, 25, or 50 ppm.	Mutagenic

Hayes (1982) reported on acute toxic effects of carbaryl in a human. Twenty minutes after ingestion of 250 mg carbaryl (approximately 2.8 mg/kg body weight), the subject experienced sudden epigastric pain, followed by profuse sweating, lassitude, and vomiting. One hour after ingestion and following a total dose of 3 mg atropine, the subject was feeling better. After a second hour, he was completely recovered.

2.5.1.2 Intraperitoneal Toxicity. Carbaryl has been reported to have a low acute intraperitoneal toxicity in studies with rats, mice, and rabbits. Acute intraperitoneal LD_{50}'s for carbaryl, of unspecified purity, reported in rats, mice, and rabbits were 200 mg/kg, 25 to 29 mg/kg, and 223 mg/kg, respectively (Hayes 1982).

2.5.1.3 Dermal. Carbaryl (purity unspecified) is very low in acute dermal toxicity. The acute dermal LD_{50} for carbaryl was reported to be greater than 4 g/kg in rats (Gaines 1969, in Kuhr and Dorough 1976). According to U.S. Environmental Protection Agency toxicology guidelines, this toxicity range places carbaryl into Toxicity Category IV, warranting its use with caution (Ashton 1982).

Vandekar (1965, in Hayes 1982) reported on the effects of dermal exposure of humans to carbaryl. Carbaryl (85 percent wettable powder) as a 5 percent spray was applied by 10 applicators inside of 16 houses at the rate of 2 g on each square meter of space. The application required 6 hours. The only clinical effect observed among the applicators was a skin rash in a applicators whose back was splashed accidently with the carbaryl. The spraymen showed no increase in excretion of 1-naphthol, a metabolite of carbaryl, but 63 humans who were sampled 1 week after spraying exhibited a significant increase in excretion of 1-naphthol.

2.5.1.4 Intravenous Toxicity. Carbaryl (purity unspecified) has exhibited high acute intravenous toxicity to rats as indicated by low reported LD_{50} values. The acute intravenous LD_{50} for carbaryl was reported to range from 24 to 42 mg/kg in rats (Hayes 1982).

2.5.1.5 Eye Irritation. Carpenter et al. (1961) reported that carbaryl is an irritant to eyes of rabbits. When 0.5 ml of a 10 percent suspension of technical carbaryl in propylene glycol was placed onto the cornea of one eye of each of 5 rabbits, one of the eyes was mildly injured. Application of a 25 percent aqueous suspension of carbaryl caused no injury, but 50 mg of the dust caused traces of corneal necrosis. According to U.S. Environmental Protection Agency toxicology guidelines, this toxicity range places carbaryl into Toxicity Category II, warranting its use with warning (Ashton 1982).

According to Cranmer (1986) more recent research in 1983 by Bushy Run Research Center showed that carbaryl produces inconsequential effects in the eyes of rabbits. Sevin RP-2 (23 percent) was reported to be a non-irritant after application (washed and unwashed eyes). Technical Sevin produced conjunctival irritation initially in 6 of 6 rabbits, but all were normal at 2 days. No eye irritation was reported for Sevin 80 sprayable (500 mg). Sevin XLR (43 percent) produced transient iritis and conjunctival irritation, but eyes were normal at 3 days. Sevin FR (40 percent) produced no eye irritation in another study conducted according to EPA guidelines.

2.5.1.6 Inhalation. Formulated carbaryl is mildly toxic by inhalation in guinea pigs (Carpenter et al. 1961). A group of 6 guinea pigs were exposed to carbaryl (50 percent wettable powder, average particle size of 15 µm) for 4 hours at a concentration of 390 mg/m^3. The authors observed nasal and ocular irritation among the exposed animals. Autopsies performed after 14 days disclosed healed hemorrhagic areas in the lungs. Another group of 6 guinea pigs were exposed to carbaryl (85 percent wettable powder, average particle size of 5 µm) for 4 hours at a concentration of 230 mg/m^3. During the 14-day observation period, the animals showed an initial slight weight decrease but regained their pretreatment weight by the end of this interval. According to U.S. Environmental Protection Agency toxicology guidelines, these toxicities place formulated carbaryl into Toxicity Category IV, warranting its use with caution (Ashton 1982).

2.5.2 Subchronic Toxicity

Subchronic toxicity tests with carbaryl have been reported in rats for both ingestion and inhalation routes of exposure and in humans for ingestion.

Groups of 10 rats of each sex were fed carbaryl in the diet at concentrations of 0, 1,500, or 2,500 ppm for 96 days (Carpenter et al. 1961). An increase in the kidney weight of females at both dosages was reported. In addition, a minor degree of diffuse, cloudy swelling of kidney tubles were observed in 4 of the 10 females fed 2,500 ppm of carbaryl. The authors speculated that the apparent difference in response between sexes occurred because the dose for females was about 30 percent greater than that for males at the same dietary intake.

Carbaryl was ingested daily for 6 weeks at doses of 0.06 mg/kg by 5 men or 0.12 mg/kg by 6 men (Wills et al. 1968). Test results among the subjects for bromsulphalein, EEG, plasma and erythrocyte ChE, complete blood count, and blood chemistry were normal at both dosages. Urine chemistry, however, showed abnormality attributable to carbaryl at the higher dose. At this dose, a decrease in the ratio of amino acid to creatinine nitrogen was reported for the urine. This altered ratio was interpreted as an indication of a slight, reversible decrease in the ability of the proximal convoluted tubules of the kidney to reabsorb amino acids (Hayes 1982).

An unspecified number of rats were exposed to an 85 percent wettable powder formulation of carbaryl at a concentration of 10 mg/m^3 for 7 hours/day, 5 days/week, for a total of 90 inhalation periods (Carpenter et al. 1961). No mortality nor grossly visible injuries were observed among the exposed animals.

2.5.3 Chronic Toxicity

Chronic toxicity data for carbaryl have been reported for dietary and oral studies in rats. Carpenter et al. (1961) reported a 2-year dietary study with carbaryl in rats in which five groups of 40 rats (20 males and 20 females) were administered carbaryl in the diet at concentrations of 0, 50, 100, 200, or 400 ppm for 2 years. No toxic effects were seen in rats fed concentrations of up to 200 ppm carbaryl. Among the animals fed 400 ppm carbaryl in the diet for 2 years, there was a cloudy swelling of hepatic cords located around the central veins.

Three groups of 48 rats (24 males and 24 females) were administered carbaryl orally (method of administration is not clear) at 7, 14, or 70 mg/kg for as long as 1 year (Shtenberg and Rybakova 1968, in U.S. Department of Health, Education, and Welfare 1976). At all doses, functional and structural changes of the pituitary gland and impairment of thyroid and gonadal function were reported.

2.5.4 Reproductive and Teratogenic Effects

The U.S. Environmental Protection Agency, in an undated report, concluded that carbaryl has low reproductive or teratogenic effects in the mammalian species tested with the exception of the dog. A dog study was evaluated by the agency and was judged not to meet current scientific standards due to insufficient number of animals in the dose groups and presence of maternal toxicity at all doses tested.

Orlova and Zhalbe (1968, in Weil et al. 1972) reported a study of reproductive effects of carbaryl in rats following administration by peroral intubation daily for 12 months at 2, 5, or 15 mg/kg/day prior to mating. The NOEL for reproductive effects in this study was reported to be 2 mg/kg. At the higher doses (5 or 15 mg/kg), reproduction was reported to be reduced although details of the study were not provided.

Several three-generation reproduction studies have been reported for rats and gerbils. A reproduction study in rats was conducted following dietary feeding of carbaryl at concentrations of 0, 2.5, or 10 mg/kg for three generations (Weil et al. 1972). No statistically significant dose related effects were observed in terms of fertility, gestation, viability of pups, or lactation. In another reproduction study, Osborne-Mendel rats were fed carbaryl in the diet at maternally toxic concentrations of 2,000, 5,000, or 10,000 ppm (equivalent to 100, 250, or 500 mg/kg body weight/day) for three generations (Collins et al. 1971). All doses caused adverse reproductive effects. At 10,000 ppm concentrations, decreases were observed in the fertility, viability, and lactation indices in all litters. At 5,000 and 10,000 ppm concentrations, decreases were observed in survival index and in the ratio of number of animals weaned per number of litters. At all three dose levels, a dose-related decrease was observed in weanling weights. In another study by Collins et al. (1971), Mongolian gerbils were fed carbaryl in the diet at maternally toxic concentrations of 2,000, 4,000, 6,000, or 10,000 ppm for three generations. At both 6,000 and 10,000 ppm concentrations, decreases were reported in the viability and survival indices of the litters and in the average number of animals weaned per litter.

Carbaryl was reported to be not teratogenic in studies with rats, monkeys, mice, and hamsters. Six pregnant Harlan Wistar rats were administered carbaryl (99.8 percent pure) in the diet at 0, 20, 100, or 500 mg/kg body weight from pregnancy through weaning (Weil et al. 1972). Carbaryl did not produce teratogenic responses in this study. Daily gastric intubation of carbaryl (purity unspecified) at 2 or 20 mg/kg body weight throughout the gestation period in monkeys caused higher rate of abortion among treated animals compared to the control group (Dougherty et al. 1971). However, examination of the live infants and some of the aborted fetuses in this study indicated that carbaryl was not teratogenic. Coulston et al. (1974) administered carbaryl by gavage to Rhesus monkeys at doses of 0.2, 2.0 and 20 mg/kg/day, administered on days 20 through 38 of gestation. After birth, young were monitored for a year. No signs of maternal toxicity were noted. Two young of the 20 mg dose group had low birth rates, but no other signs of fetotoxicity were noted.

Carbaryl was administered daily to CF-1 mice at doses of 0, 100, or 150 mg/kg body weight by gavage or at concentrations of 5,660 ppm in the diet (equivalent to 1,166 mg/kg body weight) from days 6 through 15 of gestation (Murray et al. 1979). No teratogenic responses due to administration of carbaryl were reported in this study. Carbaryl was reported not teratogenic in golden Syrian hamsters given carbaryl orally in a gelatin capsule at 125 or 250 mg/kg body weight from days 12 through 20 of gestation (Robens 1969).

Teratogenic effects of carbaryl in dogs is uncertain since no dose-response relationship of carbaryl was apparent in a study with beagle dogs (Smalley et al. 1968). In this study, beagle dogs were administered carbaryl in the diets at concentrations of 3.125, 6.25, 12.5, 25, or 50 mg/kg body weight throughout gestation. Carbaryl was not teratogenic at

3.125 mg/kg, but terata were observed at all other higher doses. However, no dose-response relationship was apparent. Specific effects that were reported included abdominal-thoracic fissures with varying degrees of brachygnathia, ecaudate pups, failure of skeletal formation, and superfluous phalanges.

All doses used in teratogenicity studies in guinea pigs were slightly below or above the maternal toxicity dose of 200 mg/kg. Therefore, teratogenic effects of carbaryl in these species are uncertain. A three-generation study was conducted in guinea pigs with daily administration of carbaryl (99.56 percent) at either 300 mg/kg in the diet or 200 mg/kg given by oral intubation (Weil et al. 1973). Carbaryl in guinea pigs was not teratogenic either by dietary inclusion or by oral intubation. Another teratogenicity study in guinea pigs was conducted in which pregnant guinea pigs were given carbaryl orally in gelatin capsule at 300 mg/kg/day during organogenesis (days 12 through 20 of gestation) (Robens 1969). This multiple treatment resulted in increased maternal and fetal mortality. Teratogenic effects that were observed were skeletal defects that occurred mostly in cervical vertebrae.

Results of teratogenicity studies of carbaryl that occurred at maternally toxic doses in rabbits were similar to those observed in guinea pigs. New Zealand rabbits were administered daily oral doses of carbaryl in gelatin capsules at 50 to 200 mg/kg daily from days 5 through 15 of gestation (Robens 1969). Carbaryl was reported to be not teratogenic in this study. In another teratogenicity study, New Zealand rabbits were administered carbaryl (99 percent pure) at 0, 150, or 200 mg/kg in cotton seed oil by gavage from days 6 through 18 of gestation (Murray et al. 1979). Carbaryl was reported to be teratogenic at both doses in this study. A significant increase in the incidence of omphalocele was observed among the offspring of rabbits given 200 mg/kg and one case was observed in the offspring of a rabbit administered 150 mg/kg. The difference in the results of Robens (1969) and this study may reflect differences in absorption of carbaryl when given by gavage in cotton seed oil compared to carbaryl administered by gelatin capsule. However, the teratogenic effects of carbaryl in this study are uncertain because the doses used were near the maternally toxic levels.

2.5.5 Carcinogenicity

The U.S. Environmental Protection Agency (undated) concluded that carbaryl is not carcinogenic in experimental animals based on the evaluation of 10 studies.

Two carcinogenicity studies of carbaryl were conducted in mongrel male rats (Andrianova and Alekseev 1970, in U.S. Department of Health, Education, and Welfare 1976). In one study, 48 rats were treated by a subcutaneous implantation of a paraffin capsule containing 20 mg of carbaryl. The animals were subsequently observed for 22 months. The control group consisted of 48 rats. Two of the 10 treated rats that survived for 22 months had fibrosarcoma in an unspecified location. In a second study, 60 rats were given carbaryl at 30 mg/kg twice a week for 22 months (whether by incorporation into their diet or by intubation is not clear). The control group consisted of 48 rats. Examination of 12 survivors (survival rate was unspecified) from the treatment group showed three fibrosarcomas and one osteosarcoma. In both studies, one fibrosarcoma occurred among 46 surviving control animals. Although the authors concluded that carbaryl could produce tumors in rats, the results of these studies are not conclusive because of the high mortality in the treatment groups and the absence of information on how the controls were treated. In addition, it is not clear whether there was 2-naphthyl N-methylcarbamate (2-naphthyl isomer of carbaryl) in the test substance. This isomer has been reported to have caused cancerous tumors in rats and mice when administered orally or intravenously (U.S. Department of Health, Education, and Welfare 1976).

Carpenter et al. (1961) reported the results of a 2-year feeding study of carbaryl with CF-N rats. Groups of 20 rats of each sex were fed carbaryl in the diet at doses of 0, 2, 4, 8, or 16 mg/kg for 2 years. The incidences of tumors (types unspecified) among the treatment groups was not different from that of the controls.

Lijinsky and Taylor (1977, in Sternberg 1979) administered carbaryl by gavage to pregnant rats either with or without a 4 percent sodium nitrite solution. The total dose of carbaryl was 300 mg administered over a 10-day period. Carbaryl alone or carbaryl administered with sodium nitrite resulted in no significant incidence of malignant tumors when compared to historical controls.

Innes et al. (1969, in International Agency for Research on Cancer 1976) reported two carcinogenicity studies in which carbaryl was administered to two strains of mice, (C57BL/6 x C3H/Anf)F_1 and (C57BL/6 x AKR)F_1, by daily oral administration in one study and by single subcutaneous injection in the other study. In the first study, groups of 18 mice of each sex and strain were initially administered carbaryl by stomach tube at a daily dose of 4.64 mg/kg in gelatin from 7 days of age through 4 weeks of age. Subsequently, the mice were administered a dose of 14 mg/kg until they were approximately 78 weeks of age. Groups of an unspecified number of control mice of each sex and strain were either untreated or had received gelatin only. Tumor incidences among the surviving mice in the four treatment groups did not differ significantly from control mice in any sex-strain subgroups or in the combined sexes of either strain. In the second study, groups of 18 mice of each sex and strain were administered carbaryl by single subcutaneous injection at a dose of 100 mg/kg in dimethyl sulfoxide from 4 weeks of age up to 78 weeks of age. Groups of an unspecified number of control mice of each sex and strain were either untreated or had received an injection of the dimethyl sulfoxide only. Tumor incidences among surviving mice in the four treatment groups did not differ significantly from that of control mice in any sex-strain subgroups or in the combined sexes of either strain. The International Agency for Research on Cancer (1976), however, noted that a negative result obtained after a single subcutaneous injection may not be an adequate basis for discounting carcinogenicity of carbaryl.

Carpenter et al. (1961) conducted a carcinogenicity study with carbaryl in male mice of two strains, A/JAX and C3H. In this study, groups of 30 3 month old mice were given weekly subcutaneous injections of 10 mg of carbaryl (equivalent to approximately 400 mg/kg body weight for a 25 g mouse) in 0.2 ml agar suspension per animal during a 5-month period. An unspecified number of control mice were either untreated or received an injection of 0.25 percent agar solution only. The incidence of lung tumors among treatment groups did not differ significantly from that of controls.

A pulmonary-tumor-induction bioassay of carbaryl was conducted in strain A/He mice by Shimkin et al. (1969, in U.S. Department of Health, Education, and Welfare 1976). In this study, 16 7- to 9-week old male mice were given 12 intraperitoneal injections of carbaryl in tricaprylin over a 4-week period. The total dose of carbaryl administered was 6 mg/animal. Three control groups of 32 mice each were injected with water, with the tricaprylin vehicle, or were left untreated. The animals were observed for 20 weeks after the last injection. The animals were subsequently killed and their lungs were examined microscopically for tumors. The incidence of lung tumors among treatment groups was classified as marginal. However, the U.S. Department of Health, Education, and Welfare (1976) noted that it is difficult to draw any conclusion as to the potential for carcinogenicity of carbaryl from this study, since it is unclear whether the test compound was 2-naphthyl isomer of carbaryl or carbaryl. As indicated earlier, the 2-naphthyl isomer of carbaryl was reported as being a carcinogen in rats and mice.

2.5.6 Neurotoxicity

Neurotoxic potentials of carbaryl have been demonstrated in several studies with rats, monkeys, and swine.

Anger and Wilson (1980) administered carbaryl (99.9 percent pure) to each of 6 rats by intraperitoneal and intramuscular injections of 1, 3, 5, or 10 mg/kg body weight for 1 or 2 times/week for a period of 6 months. Each rat served as its own control. There were significant decreases in performance in terms of response rate among animals following 3, 5, or

10 mg/kg intraperitoneal injections and 5 or 10 mg/kg intramuscular injections of carbaryl.

Tachibana et al. (1973, in Kuhr and Dorough 1976) demonstrated neurotoxic effects among rats following oral administration of carbaryl. Rats were fed carbaryl of an unspecified purity in the diet at concentrations of 100, 500, or 2,500 ppm for 35 days. There was a a progressive decrease in the ability of the exposed animals to avoid electrical punishment.

Desi et al. (1974) demonstrated neurotoxic effects among rats following oral administration of carbaryl. Male Wistar rats were fed carbaryl of an unspecified purity at concentrations of 100 or 200 ppm in the diet (equivalent to 10 or 20 mg/kg body weight) for 50 days. Eight animals each were used for control and for the two treatment groups. Carbaryl was reported to have several adverse effects on the behavior and neurophysiological functions of exposed animals. The process of learning in 200 ppm group and the performance of previously learned tasks in both groups was adversely effected after 50 days treatment. Electroencephalographic patterns showed an increase in fast and slow wave components under resting and light-stimulated conditions in both groups fed dietary carbaryl. An inhibition of acetylChE activity in various parts of the brain was reported in the group administered 200 ppm. Mild, but permanent and increasing, functional deviations of the central nervous system were also reported among treated animals.

Anger and Setzer (1979, in Miller 1982) reported that monkeys exposed to carbaryl exhibited an increase in both the number of errors and the time required to finish a session in a repeated acquisition task. Details of the experiment in terms of route of exposure and dose were unspecified, however.

Two neurotoxicity studies were conducted in swine (Smalley et al. (1969 and Smalley 1970, in Kuhr and Dorough 1976). A single oral administration of carbaryl at a dose of 1 or 2 g/kg (exposure time not specified) caused salivation, central nervous system depression, muscle tremors, and cyanosis. Dietary feeding of carbaryl at a concentration of 150 to 300 ppm/day for as long as 12 weeks resulted in a progressive myasthenia, incoordination, ataxia, and clonic muscular contractions. Continued exposure of these animals to carbaryl resulted in paraplegia and prostration.

2.5.7 Mutagenicity

Carbaryl appears to act as a weak mutagen in some mutagenic test systems. Wuu and Grant (1967) reported mutagenicity tests of carbaryl in barley seeds (Hordeum vulgare). Carbaryl applied at a concentration of

1,000 ppm for 12 hours on sprouted barley seeds, induced chromosomal aberrations of 0.55 and 2.9 percent in the C-1 and C-2 generations, respectively. The untreated control sprouted barley seeds, however, showed chromosomal aberrations of 0.22 and 0.49 percent in the C-1 and C-2 generations, respectively. Carbaryl (84 percent pure) at a concentration of 20 to 80 μg/ml culture medium was reported to inhibit mitosis and spindle fibre formation in human embryonic fibroblasts (Vasilos et al. 1972, in International Agency for Research on Cancer 1976).

In several other test systems, however, carbaryl did not demonstrate mutagenicity. Carbaryl was not mutagenic in *Salmonella typhimurium* strain LT12 (Regan et al. 1976 in International Agency for Research on Cancer 1976). Carbaryl was also not mutagenic in *S. typhimurium* TA98, TA100, TA1535, or TA1537 strains in the presence of a rat-liver microsome induced by Arochlor (McCann et al. 1975, in International Agency for Research on Cancer 1976). In both of the above studies, concentrations of the test substance were not specified.

Carbaryl was not mutagenic in *Escherichia coli* (Ashwood-Smith et al. 1972, in International Agency for Research on Cancer 1976) and in *Saccharomyces cerevisiae* (Siebert and Eisenbrand 1974). It was, however, reported to be mutagenic in *Drosophila melanogaster* (Brzeskij and Vaskov 1971, in International Agency for Research on Cancer 1976). Test conditions for these studies were not specified.

Weil et al. (1973) conducted a dominant-lethal test for carbaryl in rats. Carbaryl was administered at doses of up to 200 mg/kg/day in diet or up to 100 mg/kg/day by oral intubation until 32 weeks of age. No dominant lethal mutagenicity was caused by carbaryl.

Epstein et al. (1972) also reported that no dominant lethal mutagenicity was caused by carbaryl in mice. In this study, 10 male mice each received daily doses of carbaryl at 50 or 1,000 mg/kg for 5 days. One mouse in the group dosed at a rate of 1,000 mg/kg died. Each of the 19 surviving males was caged for mating with 3 untreated virgins each week for 8 weeks. The females were subsequently killed on about the 13th day of gestation and the total embryo implants in females mated with treated males and in females mated with untreated control males were compared. The authors reported no dominant lethal mutagenicity of carbaryl in this study.

Usha Rani et al. (1980) reported results of *in vivo* cytogenetic testing and of host-mediated assay for carbaryl in Swiss albino mice. In *in vivo* cytogenetic testing, mice were treated orally with carbaryl at a dose of 146 mg/kg. Each animal received the dose in two equal installments with a 24-hour interval between the two administrations. Six hours after the second dose, the animals were killed and the bone marrow was removed for micronucleus test. No significant increase in the frequency of micronuclei was observed in the treated group compared to the control group. In the host-mediated assay, mice were administered an oral dose of carbaryl 483 mg/kg, administered in 3 equal installments for 3 days. One day after the last treatment, the mice were injected intraperitoneally with *S. typhimurium* G46 strain. An increase in the frequency of revertants in the carbaryl-treated animals was not significantly different from that of the control group.

2.5.8 Toxicological Properties of N-nitrosocarbaryl

N-nitrosocarbaryl, is formed by reacting carbaryl with nitrous acid (Elespurn and Lijinsky 1973). Because formation of N-nitrosocarbaryl in the stomach is expected and because it is a metabolite of carbaryl, the potential health hazards from exposure to N-nitrosocarbaryl have been evaluated in rats. The results of the toxicity tests are described in this section and were summarized in table 2-3 and appendices E-1 and G-1.

Eisenbrand et al. (1975) administered single subcutaneous injections of N-nitrosocarbaryl suspended in Livio oil to 8 Wistar rats of each sex at a dose of 1 g/kg body weight. The control groups, 8 animals of each sex, received only Livio oil. N-nitrosocarbaryl was reported to induce local sarcoma at the site of injection. By day 450, 14 of 16 rats had died.

Lijinsky and Taylor (1976) administered N-nitrosocarbaryl by gavage to 15 male and 12 female Sprague-Dawley rats of 8 to 10 weeks of age. The 15 male rats received N-nitrosocarbaryl twice a week for 20 weeks for a total dose of 300 mg. The 12 female rats received N-nitrosocarbaryl once a week for 10 weeks for a total dose of 50 mg. The animals were subsequently observed for 120 weeks. Male rats died earlier than female rats and the incidence of squamous stomach tumor among males and females were 47 and 75 percent, respectively.

In another study reported by Eisenbrand et al. (1975), 37 Wistar rats of both sexes were given single oral doses of N-nitrosocarbaryl, suspended in starch paste, by stomach tube at doses of 200 to 1,500 mg/kg. Twenty-two untreated rats of each sex served as control. N-nitrosocarbaryl did not induce tumors 21 months after treatment.

Mutagenicity of N-nitrosocarbaryl was examined in 5 strains of *Salmonella typhimurium* (Blevins et al. 1977). Dose ranges of N-nitrosocarbaryl used were 0.00115, 0.0115, 0.115, 1.15, or 11.5 μg/plate. The test substance was mutagenic in both spot and plate incorporation tests. N-nitrosocarbaryl was also reported to be mutagenic in *Saccharomyces cerevisiae* (Siebert and Eisenbrand 1974). In this study, N-nitrosocarbaryl induced mitotic gene conversions at concentrations of N-nitrosocarbaryl of 0, 1, 5, 10, 25, or 50 ppm.

3.0 Environmental Fate

Carbaryl is a widely used agricultural pesticide throughout the United States and much of the rest of the world. As such, there is a considerable potential for environmental exposure to carbaryl and its formulations. Carbaryl is taken up in plants and animals. In plants, it has been shown to have some toxic effects and to be hydroxylated into several water-soluble carbamate intermediates. In animals, it is readily absorbed and also metabolized into several water-soluble carbamate intermediates and both carbaryl and its metabolites are generally rapidly excreted. Carbaryl adsorbs to soils by means of physical adsorption mechanisms, has a moderate to short persistence in soil, and is degraded by both chemical and biological mechanisms in soil environments. In aquatic environments, carbaryl and its metabolites have a relatively short persistence.

3.1 Bioaccumulation and Metabolism

Carbaryl is absorbed and metabolized in both animals and plants to several carbamate intermediates. Studies on the fate and behavior of carbaryl and its formulations in plants and animals are summarized below.

3.1.1 Plants

Studies on the fate and behavior of carbaryl and its formulations in plants have been primarily concerned with three issues, the toxic effects of carbaryl to plants, the uptake of carbaryl and its persistence in plants, and the potential for carbaryl to be metabolized by plants and by fungi. This section briefly reviews the scientific literature dealing with these three concerns.

Several studies have indicated toxic effects of carbaryl in plants. Carbaryl formulations have been used as a fruit thinning agent in apple orchards to improve the size, color, and flavor of the remaining fruit and may act primarily by reducing the rate of transport of the plant hormone, indolecetic acid (Ebert and Bangerth 1982). Hamlen and Henley (1976) reported moderate to severe injury to 2 of 10 species of tropical foliage plants that were treated with an 80 percent formulation of Sevin. In this study, carbaryl was applied four times at weekly intervals as an insecticide and miticide to 10 species of tropical foliage plants the recommended concentration of 1 lb a.i./100 g of water and at 2 lb a.i./ 100 g, twice the recommended concentration. At the recommended concentration, there was moderate injury in the form leaf deformity in one plant species. At twice the recommended concentration, there was severe injury to one species and moderate injury to a second species, both exhibiting leaf deformities. In addition, a powdery residue was observed on plants of all 10 species treated with twice the recommended concentration of the sevin formulations. Eid et al. (1971) exposed seeds of cotton, peas, rice, maize, kenaf, and radish to 1, 5, or 10 percent solution of an 85 percent wettable powder formulation of carbaryl. There was a slight increase in the rate of germination of most species of seeds, particularly at a concentration of 1 percent of formulation, that was attributed to the fungicidal side-effects of the insecticide, while at a concentration of 10 percent, there was some inhibition of seed germination, particularly among seeds of maize and rice. At concentrations of 1 and 5 percent carbaryl formulation, there was an increase in the growth of rootlets and plummules of rice seeds and at 1 percent an increase in the growth of rootlet and plummule of pea seeds. Carbaryl at concentrations of 100 to 1,000 ppm a.i. resulted in a reduced rate of germination and elongation of corn pollen compared to controls on an artificial medium, with germination of treated pollen ranging from 15.6 to 32.4 percent compared to 62.0 percent for controls in distilled water (Gentile et al. 1973). Single or multiple applications of carbaryl to the silks of corn in this study resulted in malformed and/or missing kernels.

A number of studies in the literature have examined the residues and persistence of carbaryl in and on plants after its application. Ferreira and Seiber (1981) treated rice seedlings in the laboratory with carbaryl (Sevin 4F) by root-soak or by foliar spray, both with a 500 to 800 ppm aqueous solution. They reported that the material rapidly translocated to the leaves, with residues moving to the leaf exterior by guttation from which they were volatilized. Of the initial residue of carbaryl, 22 percent was lost by root exudation and 4.2 percent by volatilization.

Narian et al (1978) measured mean residues of carbaryl in selected organs of seven home garden vegetables following 13 applications at weekly intervals to run-off of a solution of 4.0 g a.i./l of a 50 percent wettable powder formulation. Residues ranged from less than 0.1 ppm or below detectable levels in most samples to 6.55 ppm in the foliage of squash.

Residues of carbaryl were measured on forest foliage 1 to 2 hours after application through 63 days post-application following aerial spray of Sevin-4-Oil diluted 1:1 with diesel oil and applied at a rate of 1 lb/0.5 g/acre on three 1000 acre plots (Pieper 1979). Initial average residue levels were 70, 168, 30, and 138 ppm for grass, geranium, aspen, and Douglas-fir, respectively. Sixty-three days after application, residues had declined to less than 0.1 ppm on grass and geranium, and to 0.5 and 3.8 ppm on aspen and Douglas-fir, respectively. The half-lives of these residues were empirically estimated to be 8, 3, 8, and 4.5 days, respectively in grass, geranium, aspen, and Douglas-fir.

Several other studies have addressed the behavior of carbaryl in trees either in forest environments or in cultivated fruit and/or nut trees. Sundaram and LeCompte (1975) measured residues of carbaryl on white pine shoots following aerial application of Sevin 4-Oil at a rate of 1 lb a.i./acre (1.12 kg/ha) for control of white pine weevil. Maximum residues of 12.9 ppm on the surface of the shoots and of 6.2 ppm from the tissues, based on live weight of the plant material, were reported one hour after application. Fifteen days after spraying, these residues had declined to 2.0 ppm on the surface and 2.8 ppm in the tissues and at 90 days there were no detectable surface residues of carbaryl and only traces in the plant tissues. Gupta et al. (1980) reported that initial carbaryl residues were 36.55 and 29.87 ppm on leaves of neem and ber trees, respectively, following application of a 0.2 percent suspension of carbaryl at a rate of 5 l/tree. These residues had dissipated by 90 percent within 21 and 15 days for the two types of tree, respectively and the authors concluded that since deposits were below 100 ppm, the leaves could be safely fed to cattle.

The behavior of carbaryl in and on agricultural food crops has been evaluated by several authors. Singh et al. (1981) reported a half-life of 2.20 to 3.64 days for carbaryl residues in sugarcane foliage treated with Sevinmol-40LV and Sevin 50WP at a rate of 0.67 and 1.12 lb a.i./acre (0.75 and 1.25 kg/ha) with two treatments at an interval of 15 days. Residues of 79.82 to 208.77 ppm were reported for deposition in and on foliage immediately after the second application of carbaryl formulations. Argente and Heinrichs (1983) applied an 85 percent wettable powder formulation to rice in field plots at a rate of 0.67 lb a.i./acre (0.75 kg/ha). Maximum residues of 33.8 ppm were reported for the rice sampled 2 hours after application of carbaryl and declined to less than 10 ppm after 5 days.

Fjelddalen and Renvall (1974) reported data on a cooperative study of pesticide residues in field crops in Denmark, Finland, Norway, and Sweden. Carbaryl was applied to apples in Denmark before harvest at a concentration of 0.125 percent a.i. Residues in the apples were reported to be 0.9 ppm 1 day after application, 0.5 to 0.6 ppm 7 to 9 days after application, 0.03 ppm 60 to 79 days after application, and less than 0.02 ppm (limits of detection) 87 to 90 days after application.

Carbaryl, at a concentration of 0.05 percent, was applied to cabbage plants at a rate of 375 l/ha (Agnihotri et al. 1980). Residues in and on the cabbage plants were reported to be 9.4 ppm within 1 hour, declining to 6.8, 3.2, 1.5, and 0.75 ppm after 1, 3, 5, and 10 days, representing 92 percent loss of initial concentration by day 10.

Kavadia et al. (1978) raised crops of maize, beet root, radish, and carrot on a clay loam soil that had been treated with carbaryl 4G by drill at rates of 13.4, 26.8, or 40.2 lb a.i./acre (15, 30, or 45 kg/ha). No detectable residues of carbaryl were found in the crops grown in soil treated at any application rate.

Carbaryl bait was applied to potatoes at application rates of 1.11, 1.34, 2.01, and 3.57 lb a.i./acre (1.25, 1.5, 2.25, and 4 kg/ha) either in furrows at planting time before seed tuber placement or applied near the base of plants at first earthing time (Misra et al. 1977). No carbaryl was detected on or in potatoes sampled 136 days or 97 days after planting time application or earthing time application, respectively.

Singh et al. (1982) treated field plots of sunflowers with three formulations of carbaryl (Sevimol-40LV, 50 percent wettable powder, and 10 percent dust) at application rates of 0.67 to 2.68 lb a.i./acre (0.75 to 3.0 kg/ha). The insecticide was applied twice at an interval of 21 days with the first application at flower initiation. At harvest, seeds contained residues of 0.11 to 0.16 ppm carbaryl and seed cake contained residues of 0.09 to 0.15 ppm. Residues were below detectable limits in oil samples.

Ware et al. (1978) reported dislodgeable residues of carbaryl on cotton plants for a period of up to 4 days following application of a 4.0 lb/g emulsifiable concentrate (Sevimol) and an 80 percent wettable powder formulation, both at an application rate of 1.5 lb a.i./acre by ground sprayer. Residues were extracted from leaf discs at 0, 24, 48, 72, and 96 hours with acetone for both carbaryl formulations as well as with methylene chloride for the wettable powder formulation. Initial residues of Sevimol were 70.4 mg/m^2, declining to 9.2 mg/m^2 after 96 hours. Initial residues of Sevin (80 percent wettable powder) were 113.6 and 114.7 mg/m^2, declining within 96 hours to 14.2 and 18.8 mg/m^2.

Chawla and Kakkar (1981) applied a 50 percent wettable powder formulation of carbaryl to cotton at an application rate of 500 g a.i./acre for six applications at 2-week intervals during vegetative and boll formation stages. Residues of 0.22 ppm were reported for cottonseed and 0.08 ppm for lint. No metabolites of carbaryl were detected in either cottonseed or in the lint.

Iwata et al. (1979) applied an 80 percent wettable powder formulation of carbaryl to mature orange and lemon trees by boom sprayer at an application rate of 11.5 lb a.i./acre (12.88 kg/ha) and sampled dislodgeable foliar residues for as long as 60 days after application as well as residues in the fruit approximately 60 days after application. Persistence half-lives for carbaryl on foliage were reported to be 14 and 22 days for orange and lemon trees, respectively, with residues of 5.6 and 2.4 μg/cm^2 on orange and lemon foliage, respectively, 5 days after application of the carbaryl formulation. Five of six samples of orange pulp did not contain detectable residues of carbaryl at a detection limit of 0.01 ppm. The one detectable residue was 0.02 ppm. The lemon samples contained residues of 0.01, 0.01, 0.03, 0.06, 0.07, and 0.09 ppm. Although the specific identity of the residues was not determined, it was reported not to be the 1-naphthol metabolite.

Several studies have addressed the potential degradation of carbaryl by plants and by microorganisms, particularly fungi. Liu and Bollag (1971), Matsumura and Boush (1968), Rosenberg et al. (1979), and Sikka et al. (1975) have reported metabolism of carbaryl by microorganisms. Kuhr and Casida (1967) demonstrated that carbaryl is metabolized by hydroxylation in bean plants into the same water-soluble carbamate intermediates that are involved in carbaryl metabolism by mammals and by insects. These are the N-hydroxymethyl, 4-hydroxy, 5-hydroxy, and 5,6-dihydro-5,6-dihydroxy derivatives of carbaryl. The metabolism of carbaryl in plants was reviewed briefly by Heywood (1975).

3.1.2 Animals

The metabolic fate of carbaryl in animals has been studied in a variety of species of animals representing several taxonomic groups. Species used have included the human, guinea pig, sheep, monkey, pig, dog, chicken, cow, gerbil, mouse, rat, fly, and weevil, among others. Appendix H describes available studies on the fate and behavior of carbaryl in animals.

3.1.2.1 Absorption. Carbaryl is well absorbed by dermal, gastrointestinal, and respiratory routes. Absorption studies have been conducted in several species.

A dermal absorption study in rats was conducted by Shah and Guthrie (1983) by applying ^{14}C-naphthyl-labeled carbaryl in acetone to the shaved skin of male rats at the rate of 4 μg/cm^2 skin surface. They reported that 45.7 percent of the applied dose had penetrated 4 hours after dosing and 95.7 percent had penetrated within 5 days after dosing. Another study in rabbits was conducted by Shah and Guthrie (1977) by applying 3.4 mg of nonradioactive carbaryl and 68 μCi of ^{14}C-methyl-labeled carbaryl (99 percent pure) to 2 cm^2 area of the shaved skin of female rabbits. A blood sample was obtained by venipuncture from ear veins at 1, 5, 15, and 30 minutes and 1, 2, 3, 8, and 24 hours following the treatment. They reported that the radioactivity as carbaryl equivalent in the blood reached maximum levels (73.3 ppm) 8 hours after dosing and declined slightly to 49.8 ppm 24 hours after dosing.

Results of dermal absorption studies of carbaryl in man were reported by Feldman and Maibach (1974) and Maibach et al. (1971). These authors reported that dermal absorption of ^{14}C-labeled carbaryl (position of label unspecified) from the ventral forearm (Feldman and Maibach 1974) and the jaw angle (Maibach et al. 1971) of human was 73.9 and 69.9 percent of the applied dose after 5 days of application, respectively. Feldman and

Maibach (1974) estimated that the maximum absorption rate of carbaryl from the ventral forearm was 3 percent of the dose per hour. Maximum absorption rate of carbaryl from the jaw angle of man was not reported (Maibach et al. 1971).

Absorption of carbaryl by the gastrointestinal tract in the rat following oral administration of ^{14}C-carbonyl-labeled carbaryl was reported by Casper and Pekas (1971, in Kuhr and Dorough 1976). They reported that carbaryl was rapidly absorbed from the gastrointestinal tract since radioactive CO_2 in the exhaled air reached its peak 30 to 40 minutes after oral administration of ^{14}C-carbonyl-labeled carbaryl. Absorption of carbaryl by the small intestine and lungs of rats were conducted by Hwang and Schanker (1974). Solution of unspecified ^{14}C-labeled carbaryl (0.005 to 0.01 mM [millimolar]) were administered into the lungs or small instestine of anesthesized rats. The organs were removed at various times and assayed for amount of remaining carbaryl. Percentage of the dose unabsorbed was plotted against time and half-times of absorption of carbaryl from the lungs and small intestines were calculated. Results showed that half-time of absorption of carbaryl from the lungs and small intestine were 2.6 and 6.4 minutes, respectively.

In addition, absorption of the carbaryl by these organs per unit times was directly proportional to the dose of carbaryl administered. Rapid movement of carbaryl into the lung was also demonstrated by Blase and Loomis (1976) in their study using isolated perfused rabbit lung. They dosed isolated perfused lung with carbaryl concentrations of 20, 40, 80, and 120 µg/ml perfusate. Serial samples (5 ml) of perfusate were analyzed at 1, 5, 10, 15, 60, and 120 minutes. They showed that the lungs absorbed 26 percent of the applied dose in 15 minutes of perfusion and were very slow after this time. There was no evidence of saturation of uptake of carbaryl over the ranges of doses used. Rapid absorption of carbaryl by lung of the rat is also reported by Nye and Dorough (1976) following the intratracheal injection of ^{14}C-labeled (position of label unspecified) carbaryl. Radioactivity in the blood of rats reached its maximum in 2 to 5 minutes.

Accumulation of carbaryl in fish was demonstrated by Kanazawa (1975), who he reared freshwater fish in a water tank containing 1 ppm carbaryl for 30 days at water temperature of 23 ± 2 °C. The pH of water was 6.4 to 6.8. The amount of carbaryl in fish were analyzed at various intervals of day for 1 month. Maximum levels of carbaryl in fish reached 7.5 ppm after 1 day of exposure and decreased to 0.89 ppm after 7 days of exposure. Carbaryl's log Pow is 2.29 to 2.81, which shows a relatively low potential to bioaccumulate in fish (Saari and Goldfarb, 1986).

The rate of bioaccumulation of carbaryl in channel catfish was reported by Korn (1973). In this study, channel catfish were exposed to mixtures of ^{14}C-naphthyl-labeled carbaryl and nonradioactive carbaryl for 56 days continuously at the following doses: 0.05 mg/l or 0.25 mg/l in the water and 0.28 mg/kg or 2.8 mg/kg/week in the diet. Five fish were removed and analyzed after 3, 7, 14, 28, and 56 days of exposure. Previously exposed fish (0.25 mg/l in the water and 2.8 mg/kg/week in the diet) were placed on carbaryl-free diet for 0, 3, 8, 14, and 28 days to study retention of carbaryl in fish. Korn (1973) reported that the total residues of carbaryl and its metabolites accumulated by channel catfish during exposure for 56 days to 2.8 and 0.28 mg/kg/week in feed was 9 ng and 1 ng/g body weight, respectively. The total residues accumulated by catfish during exposure for 56 days to 0.25 and 0.05 mg/l in bath water was 11 and 2 ng/g body weight, respectively. This study shows the dose-dependency for both feed and water exposures. The carbaryl was reduced from parts per million levels in food and water to parts per billion levels in catfish. Fish exposed to 2.8 mg/kg/week by diet eliminated residues rapidly after they were placed on a carbaryl-free diet for 28 days. They retained 22 percent of the 0 day residue level. The residues remained constant for 28 days in fish previously exposed to 0.25 mg/l carbaryl in water.

Absorption of carbaryl was reported in rainbow trout exposed to ^{14}C-carbaryl in water at a concentration of 0.25 mg/l (Statham et al. 1975). Approximately 15 percent of the applied carbaryl was absorbed by the trout within 24 hours. The amount of radioactivity in bile represented 30 percent of the total quantity of ^{14}C-carbaryl taken up by the trout.

Uptake of ^{14}C-naphthyl-labeled carbaryl was investigated in seven species, including algae, clam, crab, *Daphnia*, *Elodea*, mosquito fish, mosquito and snail, in a laboratory aquatic microcosm (Sanborn 1974). Analysis of the organisms indicated that the concentration of ^{14}C-carbaryl and its metabolites in the various organisms ranged between 0.428 and 5.13 ppm. Thin-layer chromatographic analysis of each organism showed few radioactive spots whose identities were not known. None of the organisms contained carbaryl, indicating that carbaryl was not persistent in these organisms.

The absorption of carbaryl in the house fly, stable fly, boll weevil, and rice weevil was reported by Camp and Arthur (1967). After topical applications of carbaryl (2 μg/fly and 5 μg/weevil), the percentage of the applied dose absorbed within 24 hours was 56.7, 37.3, 12.2, and 3.3 for the stable fly, house fly, boll weevil, and rice weevil, respectively.

The rate of penetration of carbaryl into susceptible and resistant strains of German cockroach was studied by Ku and Bishop (1967). Both strains of cockroach were treated with 4 or 16 μg of carbaryl in 1.67 μl of acetone. Internal tissues and feces were collected at various times (6-hour and 12-hour intervals up to 108 hours) after the carbaryl treatment. The rate of penetration was based on the total amounts of carbaryl, 1-naphthol, and conjugates of 1-naphthol recovered from tissues and excreta. The susceptible and resistant strains absorbed 51.3 and 48.1 percent of the applied dose, respectively, during the first 36 hours after treatment but thereafter penetration was about equal in the 2 strains.

The absorption of carbaryl in gypsy moth larvae was reported by Ahmad et al. (1980). Following topical application of ^{14}C-naphthyl carbaryl, the penetration rate during 0 to 5 minutes, 5 to 60 minutes, 1 to 3 hours, and 3 to 12 hours were 2.8, 0.57, 0.07, and 0.02 percent/minute, respectively.

3.1.2.2 Tissue Distribution. In a rat study, three preparations (^{14}C-naphthyl-, ^{14}C-carbonyl-, or ^{14}C-methyl-labeled carbaryl) each were orally given to 4 rats/group at 20 mg/kg body weight. Seven days after the treatment, neither ^{14}C-naphthyl nor ^{14}C-carbonyl residues could be detected in animal tissues. Radioactivity in intestinal tracts, carcasses, and remaining organs (organs unspecified) of the rats treated with ^{14}C-methyl-labeled carbaryl amounted to 2 to 3 percent of the administered dose (Knaak et al. 1965).

In a rat study, ^{14}C-naphthyl-labeled carbaryl was applied to the skin of shaved rats at the rate of 4 g/cm^2 and radioactivity in various tissues was analyzed at intervals of 4, 8, 12, 24, 48, and 120 hours following the treatment. Radioactivity in the carcass decreased with an increase in time. Less than 0.1 percent of the total dose was found in the blood at any interval. Radioactivity in the liver was 0.13 percent of the total dose at 120 hours post-treatment (Shah and Guthrie 1983).

A tissue distribution study of carbaryl in both pregnant and nonpregnant rats following the intraperitoneal administration of either ^{14}C-naphthyl- or ^{14}C-carbonyl-labeled carbaryl showed that in these animals, ^{14}C declined rapidly for 1 to 2 hours post-treatment and declined at a slower rate thereafter. About 3 percent of the administered dose was in the fetus 8 hours post-treatment, indicating that carbaryl had crossed the placenta. Tissue analyses showed that fetal brain, heart, and lung contained more ^{14}C on a weight basis than maternal counterparts (Strother and Wheeler 1980). According to Cranmer (1986) carbaryl demonstrated repeatedly that it crossed the placenta; however, transfer to fetus was small.

Three chickens (mature Leghorn hens) were dosed with ^{14}C-labeled carbaryl at 10 mg/kg in a gelatin capsule (Paulson and Feil 1969). The hens were sacrificed at 48 hours after dosing and tissues were removed for ^{14}C-analysis. Total radioactivity in 7 organs (liver, kidney, gizzard, intestine, proventriculus, and pancreas) and in carcass residues of chickens 2 days after oral administration of ^{14}C-carbonyl or ^{14}C-naphthyl-labeled carbaryl at 10 mg/kg was 7.1 and 1.4 percent of the administered dose, respectively.

Another study in chickens was conducted by Andrawes et al. (1972) who fed ^{14}C-naphthyl-labeled carbaryl to mature Leghorn hens in the diet for 14 days at 7, 21, and 70 ppm carbaryl. Seven days after dosing, 0.153 and 0.05 percent of the dose (expressed as average of 3 dose levels) were recovered in the eggs and carcass, respectively. Residues reached maximum levels within 2 days in egg white and 6 to 8 days in egg yolk. After discontinuation of dosing, the half-life of residues was less than 1 day in the egg white, 2 to 3 days in egg yolk, and 5 days in the carcass.

Nine hens received daily oral dose of carbaryl at 0, 180, and 540 mg/kg body weight for a period of 60 days (Nir et al. 1966). At 180 mg/kg body weight, 1 to 2 ppm carbaryl equivalent appeared in fatty tissues on the 14th day of administration, and this level did not increase in spite of the continued feeding of carbaryl for as long as 60 days. At 540 mg/kg body weight, residue levels no greater than 1 to 2 ppm carbaryl equivalent were found in the muscle after 5 weeks of administration. No detectable carbaryl or its naphthol metabolite could be found in the liver, gizzard, or heart muscle of the hens at either level of administration of carbaryl.

Hens were treated with carbaryl by dipping in a 0.5 or 1.0 percent water suspension of carbaryl (Ivey et al. 1983). Residues of carbaryl and two of its metabolites, 1-naphthol and N-hydroxycarbaryl in eggs reached maximum levels 5 to 7 days after dipping. The levels of carbaryl, 1-naphthol, and N-hydroxycarbaryl were 0.08 to 0.14, 0.12 to 0.20, and 0.06 to 0.12 ppm, respectively. Although residue levels in eggs were detectable 56 days post-treatment, they did not exceed 0.5 ppm egg tolerance for carbaryl and its metabolites.

Oral administration of 2 g of ^{14}C-carbonyl-labeled carbaryl to a lactating cow resulted in the radioactive residues in milk of 1 percent of the administered dose (Baron 1968) at 9 and 10 hour post-treatment. The major component (85 percent of the radioactive residues) in milk consisted of lactose, a natural component of milk. These data indicate that following hydrolysis of the ^{14}C-carbonyl-labeled carbaryl, a small quantity of ^{14}CO2 was incorporated into lactose.

Bees were fed 2 ppm carbaryl in honey or sugar solution for 56 days (Winterlin et al. 1973). Carbaryl residue levels at the end of 56 days in honey, bee bread, and bees ranged from 0.025 to 0.05 ppm, 0.04 to 0.08 ppm, and 0.016 to 0.02 ppm, respectively. Following the termination of the carbaryl feeding, detectable amounts of carbaryl residues in honey, bee bread, and bees were found even up to 64 days post-treatment.

A tissue distribution study of carbaryl in fish was conducted by Verma et al. (1977). They dosed fish in a tank with 8.42 mg carbaryl/ml. The accumulation of carbaryl was highest after the treatment for 4 days as compared to after 8, 15, 30, and 45 days treatment. The amounts of carbaryl were highest in gills (6.6 to 6.8 ppm) and least in the intestine (3.0 to 3.3 ppm) while the amounts found in liver, kidney, and muscles were between these two extremes. Carbaryl was found only in the gills in the range of 0.32 to 0.44 ppm 45 days post-treatment.

Kanazawa et al. (1975) monitored the bioaccumulation of carbaryl in algae, duckweed, snail, crayfish, and catfish following exposure to carbaryl in the water of an aquatic environment for 20 days. The maximum concentration of carbaryl in the water was 16 ppb after 7 days and decreased to 9.5 ppb after 20 days. Concentrations of carbaryl in the dried tissue of the algae, duckweed, snail, crayfish, and catfish were 37.9, 34.2, 2.81, 2.48, and 1.33 ppm, respectively, after 20 days of exposure. This shows that bioaccumulation of carbaryl was high for algae and duckweed and low for snail, crayfish, and catfish.

Carbaryl residues in the fatty tissue of roe deer, field-living and forest-living, were analyzed by Krynski et al. (1982). Number and type of tissues examined were 70 brains, 80 lungs, and 56 testes from field-living roe deer, and 22 samples of the same organs were taken from forest-living roe deer. No perceptable carbaryl residue was found in brains, lungs, and testes samples from roe deer from either the forest field population.

3.1.2.3. Excretion. Most animals including rat, pig, sheep, cow, chicken, gerbil, guinea pig, mouse, and dog excreted between 30 to 94 percent of an administered dose in the urine and 1 to 15 percent of the dose in the feces within 24 hours after treatment with ^{14}C-naphthyl- or ^{14}C-methyl-labeled carbaryl (Knaak et al. 1965 and 1968, Dorough 1967, Baron et al. 1969, Paulson and Feil 1969, Knaak and Sullivan 1967).

The amount of ^{14}CO2 in exhaled air from rat, chicken, gerbil, guinea pig, and mouse treated with ^{14}C-carbonyl-labeled carbaryl was 26 to 65 percent of the administered dose, respectively showing the significant amount of hydrolysis of carbaryl in these animals (Knaak et al. 1965, Benson and Dorough 1984, and Paulson and Feil 1969).

Humans excreted 28 percent of the administered dose of carbaryl in the urine during 24 hours after the oral administration of carbaryl at 2 mg/kg body weight (Knaak et al. 1968).

Rats with a bile cannula in place were given ^{14}C-naphthyl-labeled carbaryl orally at a dose of 0.01 mg/kg body weight (Marshall and Dorough 1979). Within 2 days, radioactivity in bile, urine, and feces accounted for 45.4, 42.3, and 1.4 percent of the administered dose, respectively. A similar study was conducted by Struble et al. (1983). In this study, ^{14}C-carbonyl-labeled carbaryl at doses of 1.5, 30, or 300 mg/kg body weight were given orally. Radioactivity recovered within 12 hours after dosing in bile, urine, and feces accounted for 15 to 46 percent, 10 to 40 percent, and less than 1 percent, respectively. These findings indicate that oral administration of carbaryl to rats resulted in an enterohepatic circulation of carbaryl and its metabolites.

The rate of excretion of carbaryl was studied in susceptible and resistant strains of German cockroach by Ku and Bishop (1967). Both strains of cockroach were treated with 4 or 16 g of carbaryl in 1.67 l of acetone. Internal tissues and feces were collected at various times (6 hour and 12 hour intervals up to 108 hours) after the carbaryl treatment. The rate of excretion of carbaryl and its metabolic products was more rapid in the resistant strain than in the susceptible strain. This appears to be the contributing factor in the differences in susceptibility to carbaryl in these two strains.

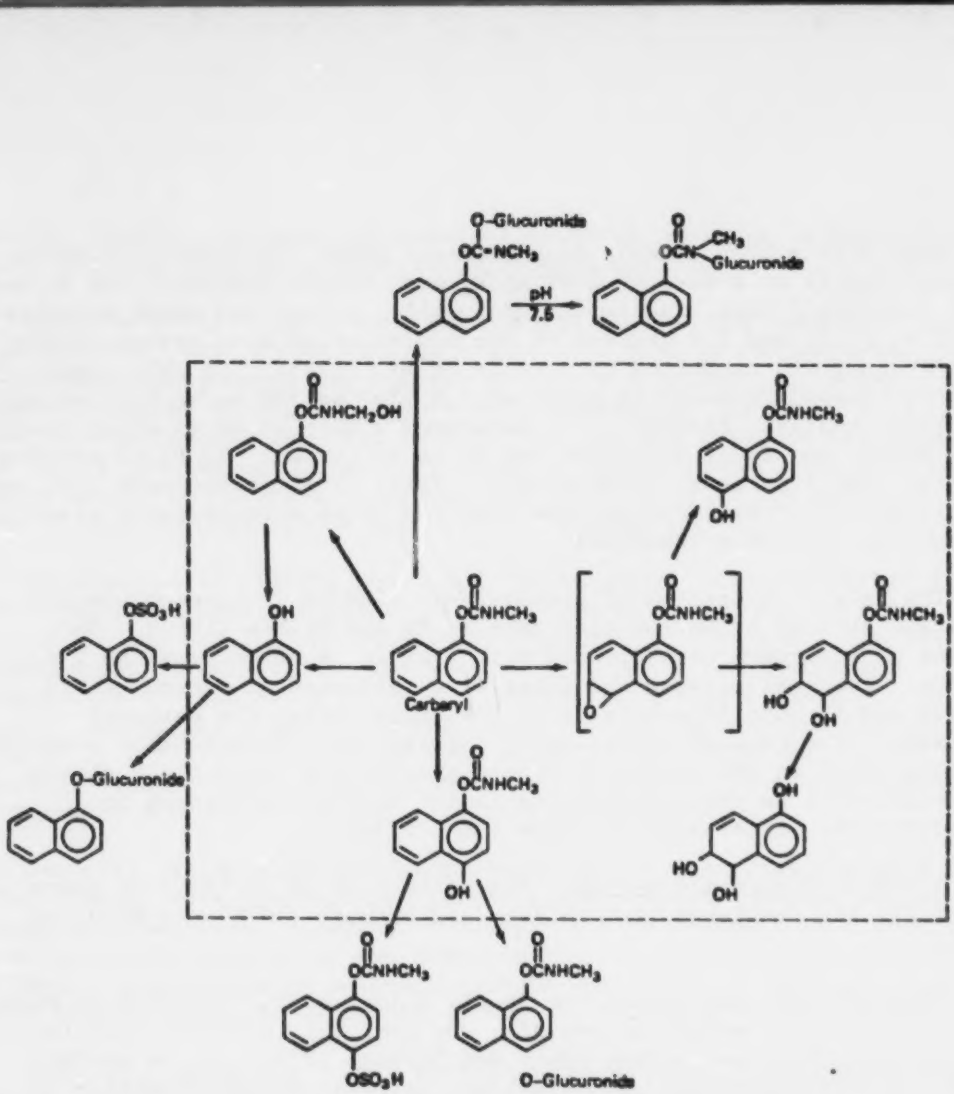
3.1.2.4 Biotransformation. The metabolism of carbaryl in mammalian system has been reviewed by both Kuhr and Dorough (1976) and by Hayes (1982).

Carbaryl does not accumulate in the animal body. Carbaryl is readily hydrolyzed in rat, sheep, guinea pig, and dog, but is fairly resistant to hydrolysis in pigs and monkey (Kuhr and Dorough 1976). It is rapidly metabolized by hydrolysis and oxidation, followed by the formation of glucuronide or sulfate conjugates and readily excreted in the urine (Environmental Protection Agency 1983).

The following diagram (figure 3-1) of the many metabolic routes for carbaryl is taken from Casarett and Duoll (1975). In humans and many other mammalian species, carbaryl is metabolized by hydrolysis and oxidation, followed by the formation of glucuronide or sulfate conjugates and readily excreted in the urine.

The following is a summary of literature reviews found in Casarette and Duoll (1975), Kuhr and Dorough (1976), and U.S. Environmental Protection Agency (1983).

Carbaryl is oxidatively metabolized by the liver to form derivatives of naphthol such as 1-naphthyl-N-hydroxymethylcarbamate, which may be subsequently hydrolized to naphthol, releasing CO$_2$ and methylamine. Other metabolic derivatives include 4-hydroxy-1-naphthyl-N-methylcarbamate and



O–Glucuronide
OC–NCH3
pH 7.5
OCN CH3 Glucuronide
OCNHCH2OH
OCNHCH3
OSO3H
OH
Carbaryl
O
HO
OH
O–Glucuronide
OCNHCH3
OSO3H
O–Glucuronide

A Metabolism of carbaryl pathways within dashed rectangle demonstrated with liver microsomes _in vitro_. Compounds outside rectangle demonstrated urinary metabolites.

Source: Casarette and Doull 1975

Figure 3-1
Examples of Metabolism of Carbamate Insecticides

the 5-hydroxy analogue along with conjugates of these materials. In addition carbaryl readily forms conjugates with amino acids (Kuhr and Dorough 1976).

A nonhydrolytic metabolism of carbaryl existed in separate preparations of radiolabeled carbaryl at three different locations in the molecule: (a) at the one position on the naphthyl ring, (b) at the carbonyl carbon, and (c) at the N-methyl carbon. Each preparation was evaluated in in vivo and in vitro experiments that demonstrated that hydrolysis of the carbamate acid ester occurred after their in vivo metabolism. These nonhydrolized hepatic metabolites when isolated included:

- 4-hydroxy-1-naphthyl-N-methylcarbamate (4-hydroxycarbaryl)
- 5-hydroxy-1-naphthyl-N-methylcarbamate (5-hydroxycarbaryl)
- 1-naphthyl-N-hydroxymethylcarbamate (N-hydroxycarbaryl)
- 5,6-dihydro-5,6-dihydroxy-1-naphthyl N-methylcarbamate. (5,6-dihydro-5,6-dihydroxycarbaryl)

The maximum rate of experimental metabolic degradation of carbaryl was achieved in the presence of microsomes with soluble extracts of rat liver and NADPH. As shown in figure 3-1 from Casarette and Duoll (1975), this metabolism includes liver microsomal hydrolysis to naphthol, followed by oxidation and urinary excretion as the sulfate or glucuronide conjugates.

Intermediate metabolites that have been isolated and identified from intact rodents include the following:

- 1-naphthol
- methylcarbamic acid
- 4-hydroxycarbaryl
- 5-hydroxycarbaryl
- 5,6-dihydro-5,6-dihydroxycarbaryl
- 1,3,4-trihydroxynaphthalene
- 1,5,6-trihydroxynaphthalene

Struble et al. (1983) gave rats oral doses of ^{14}C-carbonyl-labeled carbaryl and ^{14}C-naphthyl-labeled carbaryl. Metabolites collected from a bile fistula included 5,6-dihydro-5,6-dihydroxycarbaryl glucuronide, carbaryl conjugates, and conjugated isomers of hydroxycarbaryl.

After excision from rats, the cranial region of the small intestines caudal to the bile duct was promptly everted and incubated for 2 hours in a Krebs-Ringer phosphate medium containing 10^{-5} M carbaryl. Metabolites of carbaryl identified included 4- and 5-hydroxycarbaryl, naphthol, various hydrogenated and hydroxylated naphthols, and glucuronides. Following intravenous injection of carbaryl, 1-naphthyl glucuronide was the major metabolite found in the partial blood of the rats during 60 minutes of observation.

Liver microsomal preparations from rabbit, rat, and mouse tissues or human embryonic lung cell suspensions were incubated with carbaryl by several authors which yielded hydroxylated metabolites and 1-naphthol. Incubated ^{14}C-labeled carbaryl in a mouse liver homogenate produced thioether amino acid conjugates; detailed chemical structures were not determined.

Although carbaryl is primarily metabolized by oxidative reactions, most species also hydrolize it to the 1-naphthol. During the process these oxidation intermediates and the naphthol are conjugated to form glucuromides or sulfates. Most animal species including sheep, guinea pig, monkey, pig, and human metabolize carbaryl to 1-naphthol, CO_2 and methylamine. However, glucuronide of oxidation intermediate was not attached to the N-methylamine moiety but rather at the 5 position following the opening of one bond at the 5,6 position of the napthyl ring.

The urinary metabolites of carbaryl in sheep, guinea pig, monkey, pig and human are similar to those of the rat, although the quantity metabolized by monkeys and pigs is comparatively small. The dog produces an entirely different set of metabolites, but the dog's metabolites have not been characterized.

In the metabolism of radiolabeled carbaryl in dairy cattle, using 1-naphthyl-^{14}C, 90 percent of the 18 metabolites were water soluble, with nearly 60 percent either 5,6-dihydro-5,6-dihydroxycarbaryl or its hydrolytic product. In a second experiment, cows were fed 1-naphthyl-^{14}C in their diet up to 14 days, equilibrium of the labeled material in the cows was reached after 4 days. About 45 percent of the water-soluble products were 5,6-dihydro-5,6-dihydroxycarbaryl and its hydrolysis product. About 15 percent of the labeled material was 1-naphthol after hydrolysis and clean-up. Enzymatic hydrolysis with glusulase led to the isolation of 1-naphthol (40 percent) and 5,6-dihydro-5,6-dihydroxycarbaryl (25 percent). Clearly these latter two compounds make up a major portion of the metabolic products of carbaryl by cattle. Monoamine oxidase inhibitors led to a major decrease in the elimination of the carbaryl. This decrease was due primarily to inhibition of conjugative metabolism and oxidative degradation.

Insects (e.g., houseflies and American cockroaches) were found to use nonhydrolytic pathways for metabolizing carbaryl to water-soluble metabolites. These pathways were the same as those reported for mammalian, nonhydrolytic metabolism that involved carbaryl radiolabeled at three sites: (a) at the carbonyl carbon, (b) at the N-methyl carbon, and (c) at the 1 position on the naphthyl ring. It was presumed that these metabolites were conjugated to form water-soluble products. This presumption is extensively supported by the studies on houseflies, boll weevils, boll worms, rice weevils, cotton leaf worms, silk worms, cabbage loopers and European corn borers (Kuhr 1969, Andrawes and Dorough 1967, Camp and Arthur 1967, Kuhr and Dorough 1976). In the latter two reports, the amount of hydrolysis products usually ranged from virtually nil to about 10 percent.

The products of these metabolizing, nonhydrolytic products were predominantly oxidized compounds. Insect species that are more resistant or tolerant of carbaryl are those having less absorption, higher metabolism, and more rapid excretion. In general, attention must be given to the metabolism of 1-naphthol since it is a major hydrolysis product of carbaryl. The naphthol molecule is exceptionally resistant to ring opening, but it is readily subject to substitution.

The metabolism study of carbaryl was studied in roaches and hornworms (Shah and Guthrie 1971). The ^{14}C-naphthyl-labeled carbaryl (0.1 μg) was applied to midget tissues immersed in the buffer. In roaches and hornworms, metabolism of carbaryl was slow, and little difference in metabolic patterns were noted at 80 minutes post-treatment. Percent of radioactivity found as carbaryl and 1-naphthol, a hydrolysis product of carbaryl, in these species were 80 to 84 and 11 to 12 percent, respectively.

The metabolism of carbaryl in silkworm was reported by Moriyama et al. (1972). They treated 2-day-old 5th-instar larvae and 7-day-old 5th-instar larvae with ^{14}C-carbonyl-labeled carbaryl at 50 and 1.5 μg/g body weight, respectively. Analysis of tissues showed that the primary metabolite of carbaryl was 2-hydroxycarbaryl.

3.2 Soil

The literature on the behavior of carbaryl in soils deal with three general areas: adsorption, mobility, and leaching; persistence and residues; and mechanisms of chemical and biological degradation. In general, carbaryl sorbs physically to soil particles, has a moderate to short persistence in most soils, has been demonstrated to leach into the water table in at least one study, and has been degraded by both biological and physical mechanisms in soil.

Carbaryl sorbs to soil particles, and the process appears to be primarily one of physical adsorption rather than chemisorption (Kuhr and Dorough 1976). LaFleur (1979) studied the adsorption of several pesticides on artificially modified soils and two agronomic soils in the laboratory and reported that the adsorption ratio (amount sorbed per amount applied) decreased with increasing pesticide concentration for substrates containing organic matter, but was independent of pesticide concentration for substrates that did not contain organic material. These ratios were independent of time for inorganic substrates but increased with increasing time in organic substrates. Carazo et al. (1970) reported the 1-naphthol metabolite of carbaryl was more strongly sorbed than was carbaryl on an acid mineral soil, but that on organic soils, the parent compound and the metabolite were sorbed to the same extent. Briggs (1981) also reported that increasing organic matter in soils resulted in an increase in the adsorption of carbaryl, and that soil adsorption and leaching behavior of carbaryl could be predicted from octanol/water partition coefficients, since carbaryl is non-ionizing and thus soil organic matter is the major determinant of adsorption. Aly et al.(1980) reported carbaryl adsorption on soils increased as the temperature decreased, and that adsorption was greater on a calcium bentonite substrate, followed by a Nile alluvial soil, and a calcareous soil.

Carbaryl has a moderate to short persistence in most soil environments compared to other pesticides, with a soil half-life generally less than 2 months in most studies. Several authors have addressed the persistence of carbaryl and its formulation in a variety of laboratory and field studies.

Caro et al. (1974) measured the persistence and loss of soil incorporated carbaryl in a small (0.79-ha) watershed in Ohio. The plot had an average slope of 9.6 percent and the soil was a silt loam with an average pH of 5.2 and 1.7 percent organic matter. The plot was treated with a granular formulation at an application rate equivalent to 4.5 lb a.i./acre (5.03 kg/ha) with the material to a depth of approximately 2 inches (5 cm). Soil samples were taken 5, 12, 25, 39, 62, and 116 days after application and samples of runoff water and suspended solids were taken during each runoff event. The authors reported that only 5.77 g of a total of 4 kg of carbaryl initially applied to the soil was lost in runoff water and sediments, most of which (in excess of 90 percent) occurred during one precipitation event that occurred 19 days after application. Of the loss in runoff, 75 percent of the seasonal loss was in water and 25 percent in sediment. The pesticide was considered stable in soil sampled from various sites for 25 to more than 116 days, after which it decayed rapidly. The lag period for this decay was indicative of a primary effect of microbiological degradation. Approximately 95 percent of the applied material had disappeared within 135 days of application.

Flores-Ruegg et al. (1980) treated an organically rich and an organically poor soil with ^{14}C-labeled carbaryl and measured the residual radioactivities in these soils for 30 days following application from both nonsterile and sterile (autoclaved) samples of these soils. By day 30, residual radioactivity was 45 and 37 percent in the organic rich and organic poor soils, respectively, that had not been autoclaved. In the autoclaved soils, considerably more residual activity remained at 30 days, and was 66 and 72 percent in the organic rich and organic poor soils, respectively. Rajagopal et al. (1983) measured the residues of carbaryl and of the 1-naphthol metabolite in three soils after incubation in a flooded condition (with sterilized distilled water). Soil samples in this study received either one, two, or three applications of carbaryl in aqueous solution. In all treatments, the half-life of the applied carbaryl (based on recovery on day 0) ranged from 10 to 15 days. The disappearance time for 75 percent of the carbaryl was reported to be 20 to 26 days for samples given three applications, 28 to 30 days for samples with two applications, and greater than 30 days for samples treated with a single application of carbaryl. Carbaryl degradation was primarily by hydrolysis to 1-naphthol, with decreasing amounts of 1-naphthol being recovered with time in two of the three soils. The authors postulated that significant amounts may have been bound to humic material, particularly in soil with relatively high organic matter. Venkateswarlu et al. (1980) reported that 37 to 73 percent of applied carbaryl was recovered from samples of flooded soil in the laboratory 15 days after application of the pesticide. They further noted that degradation of carbaryl was more rapid under flooded conditions than under nonflooded conditions.

Persistence of carbaryl in field soils has been reported by several authors. Kavadia et al. (1978) applied a granular formulation of carbaryl to a clay loam soil at application rates of 13.4, 26.8, and 40.2 lb a.i./acre (15, 30, and 45 kg/ha). The soil plots were planted with either maize, beet root, radish, or carrots and the residues of carbaryl measured on days 0, 5, 10, 15, 30, 60, and 100. Five days after application, initial residues of carbaryl had been reduced by at least 50 percent in all plots but one. Soil treated with 13.4 lb/acre (15 kg/ha) and planted with maize had only lost 42.4 percent of the initial carbaryl after 5 days, the remaining plots had lost from 52.81 to 68.88 percent of the initial levels of carbaryl. The highest residues reported immediately after application were 525.9 ppm in soil treated at the highest application rate. The highest residue levels sampled from all plots decreased to 229.7, 142.9, 73.8, 33.8, 6.7, and 1.3 ppm sampled on days 5, 10, 15, 30, 60, and 100, respectively. Residues were below detectable levels in most plots by day 100 and in several plots by day 60. These results are in agreement with data provided by Gangwar et al. (1978) who applied a 4 percent granular formulation to sandy loam planted with a grain crop (bajra), at application rates of 17.9, 35.7, and 53.6 lb a.i./acre (20, 40, and 60 kg/ha). Initial residues of 140.0 to 354.5 ppm had decreased by a total of 53.01 to 31.47 percent within 30 days and 98.08 to 94.61 percent within 90 days. Residues at 90 days were reported to be 2.68 to 17.49 ppm.

LaFleur (1976 and 1980) reported a series of studies on the movement of carbaryl in a sandy loam soil. Carbaryl was applied to a field plot at an application rate of 22.7 lb/acre (25.4 kg/ha) and movement of carbaryl into the water table, at a depth of approximately 3.5 feet (1.1 m), was monitored for 16 months after application. After 16 months, the upper meter of soil contained approximately 6 percent of the applied carbaryl, with a half-life for soil loss of less than 1 month. After 4 months, there was no carbaryl detectable in the upper 7.9 inches (20 cm) of the soil layer. Detectable amounts of carbaryl were found in the water table within 2 months of application and persisted through 8 months after application. During the 16-month period, rainfall on the plot was approximately 72 inches (185 cm) representing 3.5 pore volumes of the soil in the first meter of depth.

Carbaryl is degraded in soil environments by both chemical and biological mechanisms. Aly et al. (1979) followed degradation of carbaryl in a variety of soils that were either sterilized by autoclaving or were not sterile and reported that microbiological mechanisms were of primary importance in the degradation of carbaryl in soil. In a calcareous soil with 0.41 percent organic matter, the half-life of carbaryl was 2.3 days in nonautoclaved soil and 7.3 days in sterile soil. In an alluvial soil with 2.95 percent organic matter, the half-life for carbaryl was 6.6 days in nonautoclaved soil and 18.7 days in sterile soil. Bollag and Liu (1971 and 1972) isolated several species of soil microorganisms capable of metabolizing carbaryl and reported several metabolites following degradation of carbaryl by 18 varieties of soil fungi representing 9 genera. Most of the organisms, with the exception of 2 of 3 isolates of Fusarium, Geotrichum candidum, 1 of 5 isolates of Aspergillus, and 1 of 3 isolates of Penicillium, metabolized carbaryl to 1 or more of 3 hydroxylated derivatives: 1-naphthyl N-hydroxy-methylcarbamate, 4-hydroxy-1-naphthyl methylcarbamate, or 5-hydroxy-1-naphthyl methylcarbamate. The 1-naphthol metabolite disappeared in some cultures, indicating the ability of some isolates of soil fungi to metabolize this particular metabolite. The soil fungus Trichoderma viride was demonstrated by Matsumra and Boush (1968) to have a relatively low level of activity against tritiated carbaryl.

3.3 Water

The degradation of carbaryl in aquatic systems has been reviewed by Paris and Lewis (1973). Carbaryl is stable to hydrolysis in acidic water but will undergo hydrolysis under neutral to basic conditions. Under most environmental conditions, carbaryl is not expected to persist in aquatic environments.

Aly and El-Dib (1971a, 1971b, and 1972) examined the effects of various environmental variables on the persistence of carbamates, including carbaryl, in aquatic systems. Hydrolysis of carbaryl was strongly affected by increasing the pH of the test media. The authors were able to measure hydrolysis at a pH of 7.0 and reported half-lives of 10.5 days, 1.8 days, 2.5 hours, and 15 minutes at pH levels of 7, 8, 9, and 10, respectively. Carbaryl was stable in water at acidic pH levels (below pH 7). Hydrolysis rates were increased with increasing temperatures and decreased with increasing salt contents of the media. In general, carbaryl had a low level of adsorption on kaolinite and bentonite clays. Carbaryl irradiated with two 15-watt germicidal bulbs with a peak irradiation at 254 μ had reported half-lives for photodecomposition of 60, 39, and 8 minutes at pH values of 5, 7, and 8, respectively. In Nile River water that was buffered at a pH of 7.2 and kept at room temperature of 25 ± 2 °C under aerobic conditions, carbaryl (at an initial concentration of 4.75 ppm) was rapidly degraded, with 89 percent of the total amount of material degraded within 6 days by hydrolysis to 1-naphthol by microbial biodegradation. The 1-naphthol so produced was further degraded by microorganisms.

Karinen et al (1967) followed the persistence of carbaryl and the metabolite 1-naphthol in simulated estuarine water and mud in laboratory aquaria. In water without mud, at an initial pH of 8.0, temperature of 8 °C, and salinity of 30 parts per thousand (ppt), the initial carbaryl concentration had decreased by 50 percent by 38 days, with most of the material having been degraded to 1-naphthol. At a temperature of 20 °C, the carbaryl had also completely disappeared after 17 days, and 43 percent of the parent compound had been degraded to 1-naphthol. When mud was added to the aquarium system, carbaryl and 1-naphthol in the sea water was less than 10 percent of the initial concentration within 10 days, most of the material had been adsorbed onto the sediment where it was degraded at a slower rate than in the sea water. These authors also treated mud flats in the field with an 80 percent wettable powder formulation of carbaryl at an application rate of 10 lb a.i./acre (11.2 kg/ha). Under these conditions, carbaryl persisted in the mud flats at detectable levels for as long as 42 days, but the metabolite, 1-naphthol persisted for only 1 day.

Residues of carbaryl and of its metabolite, 1-naphthol, were reported for as long as 6 days in irrigation and drainage canal water in Egypt following application along 50 meters of the bank with 1 liter of a solution containing 1 g of an 85 percent carbaryl formulation (Osman and Belal 1980). The concentration of residues of carbaryl and of 1-naphthol were not provided. The water characteristics reported included pH of 7.98 to 8.33 and 55 ppm organic and volatile matter.

Field samples of water were taken in Montanna streams during the first 3 hours after aerial application of a Sevin-4-Oil formulation, diluted with an equal volume of diesel oil, at a rate of 1 lb/0.5 g/acre (Pieper 1979). Residue levels in the streams were variable, ranging from 3 to 6, 3 to 175, 2 to 260, and 4 to 108 ppb in each of the four streams sampled, respectively. No indication was given of the residues in the streams after the first 3 hours following application of the carbaryl formulation.

The rate of disappearance of carbaryl was calculated from sampling data from streams in Maine contaminated during application of carbaryl for control of spruce budworm (Stanley and Trial 1980). Peak concentrations of 0.9 to 16.0 ppb (measured as carbaryl and 1-naphthol) were reported in brooks and 0.4 to 2.0 were reported in rivers shortly after application of carbaryl at a rate of 0.75 lb a.i./acre (0.84 kg/ha) to the watersheds. The half-life of carbaryl in these waterbodies was calculated to be 23 hours in brooks, 28 hours in rivers, and 25 hours for all of the streams combined. The authors noted that disappearance curve of carbaryl did not change significantly in slope when comparing samples taken shortly after spray application with samples taken at a later date.

Szeto et al. (1979) reported that carbaryl was degraded to 18 to 20 percent of the initial amount after 42 days in pond water (pH 7.5 to 7.8) and 37 to 40 percent of the initial amount in creek water (pH 7.0 to 7.1) after 50 days. The presence of sediment in the test water did not affect the rate of loss of carbaryl. When the water was autoclaved, however, the rate of degradation was lessened considerably, with 55 percent recovery of carbaryl after 50 days if water and sediment were autoclaved prior to treatment and incubation. Gibbs et al. (1984) followed the behavior of carbaryl (Sevin-4-Oil) for as long as 30 months following application of carbaryl at a rate of 0.75 lb a.i./acre (0.84 kg/ha). They reported maximum residues of 254 ppb in the water and 53.793 ppm in the sediment. Detectable levels of residues occurred in the water (at a detection limit of 0.06 ppb) for up to 14 months and in the sediments (6 ppb, dry weight) for as long as 16 months. The pH values of the treated ponds ranged from a minimum of 4.0 to a maximum of 7.7.

As part of a survey of surface waters in Holland, Greve et al. (1972) reported residues of carbaryl in water samples from the Rhine River. Two samples contained 0.40 and 0.20 ppb carbaryl, respectively.

Thomas et al. (1982) treated rice fields with carbaryl at application rates of 0.56, 0.84, and 1.65 lb a.i./acre (0.63, 0.94, and 1.85 kg/ha) either as a high volume spray (500 l/ha) or as a low volume spray (150 l/ha) and monitored the residues of carbaryl in the irrigation water of the rice fields 1, 4 and 10 days after carbaryl application. Residues in the irrigation water of the rice fields measured in water sampled one hour after application of the carbaryl ranged from 0.15 to 0.30 ppm in the high volume applications and from 0.07 to 0.18 in the low volume applications. By 10 days after spray, these residues had decreased to 0.02 to 0.05 ppm for the high column applications and 0.03 to 0.04 ppm for the low volume applications.

Several authors have reported on the chemical and/or biological degradation of carbaryl in laboratory studies. Liu et al. (1981) measured the rate of carbaryl under aerobic and anaerobic conditions in simulated laboratory aquatic environments in which carbaryl was introduced under abiotic (control) conditions, as a metabolite (carbaryl as the sole source of carbon and energy), or as a co-metabolite (carbaryl along with glucose and peptone). Under aerobic conditions, the half life for carbaryl degradation was 8.3, 6.8, and 3.8 days with abiotic, metabolic, and co-metabolic conditions, respectively. Under anaerobic conditions, the half-life for carbaryl degradation was 15.3, 5.8, and 4.2 days with abiotic, metabolic, and co-metabolic conditions, respectively. These data indicated that the addition of exogenous organic nutrients, simulated under the co-metabolic conditions, could significantly decrease the half-life for degradation of carbaryl in aquatic environments. Correcting for the degradation rates of carbaryl under control (abiotic) conditions, the authors reported that the biodegradation rates of carbaryl in aquatic environments would occur with half-lives of 54 and 7.5 days for aerobic conditions with metabolic and co-metabolic metabolism, respectively and would be 11.6 and 6.1 days for anaerobic conditions with metabolic and co-metabolic metabolism, respectively. The overall results of this study indicate the primary importance of abiotic processes in the degradation of carbaryl in clean aquatic environments, such as mountain streams where organic nutrients are less abundant than in other aquatic environments, and the coequal roles of both abiotic and biotic processes in the degradation of carbaryl in polluted aquatic environments.

These studies supported the data provided in 1975 by Paris et al. These authors reported that bacterial removal of carbaryl in aquatic systems was negligible, even when the bacterial population was enriched from 1 x 10^9 to 1 x 10^{11} bacteria/l. They concluded that bacterial growth was dependent upon the chemical hydrolysis of carbaryl to 1-naphthol, which in turn was used as a source of carbon by populations of bacteria.

Wolfe et al. (1978) compared the rates of hydrolysis, photolysis, and biolysis for carbaryl in aquatic systems. They calculated hydrolysis half-lives of 1,500, 15, and 0.15 days at pH values of 5, 7, and 9, respectively, assuming pseudo-first-order kinetics; a direct photolysis half-lives of 6.6 days at pH values of 5 and 7, and a biolysis half-life of longer than 3,000 days at a pH of 5 and assuming a bacterial population of

0.1 mg/l. These authors concluded that carbaryl would not persist in the aquatic environment, since hydrolysis would be very rapid at pH values greater than 7 and direct photolysis half-lives would be short at lower pH values.

4.0 Exposure and Hazard

Forest workers, visitors, and resident or transient animals are all potentially exposed to carbaryl and to its degradation products, such as 1-naphthol, following forestry applications of carbaryl. For humans and other terrestrial animals, exposures can be by dermal, inhalation, and oral (ingestion) routes. However, it must be emphasized that exposures are not necessarily correlated with actual absorption of carbaryl or any other pesticidal material. Carbaryl is expected to be absorbed through the skin and through the lungs if it reaches the alveoli. Ingestion of carbaryl will result in absorption through the gastrointestinal tract. Aquatic animals are potentially exposed by contact with waterbodies that may contain dissolved carbaryl or its metabolites.

It should be noted that, for USDA Forest Service applications, occupational exposures to carbaryl and to its metabolites are infrequent and last for relatively short periods of time. In addition, the potential for exposures are relatively limited, since a significant majority of the carbaryl used in USDA Forest Service projects is applied by aerial spray, thus reducing the potential for direct contact with spray formulations on the part of most applicators. For the most part, carbaryl that is applied by ground application techniques in USDA Forest Service projects is applied in nursery and seed orchards where public access is restricted and where animals are excluded by fencing.

The potential for release of carbaryl to surface waters and underground aquifers is low since carbaryl is not applied directly to waterbodies in USDA Forest Service applications. In addition, carbaryl has a relatively low mobility and a short to moderate persistence (two-month half life) in soil, thus reducing the likelihood for carbaryl to reach waterbodies in appreciable amounts as a result of application by the USDA Forest Service. Since the solubility of carbaryl in water is low (40 ppm at 86 °F [30 °C]), the potential for dissolved carbaryl and its metabolites to be carried into surface waters with precipitation runoff is small.

In this pesticide background statement, estimates of potential exposure to carbaryl due to USDA Forest Service applications are based upon available data from the published literature. In several of these studies, actual levels of exposure have been estimated based on field monitoring data obtained during application of carbaryl formulations and subsequent re-entry of harvesters. These studies closely simulate conditions in USDA Forest Service applications in nurseries, where workers are potentially exposed during application of benomyl and during re-entry into the treated nursery plots for weeding and transplanting nursery trees.

4.1 USDA Forest Service Carbaryl Application

Available application data for carbaryl use by the USDA Forest Service for 1983 are presented in table 4-1. These data include the number of forest workers involved in the application of carbaryl formulations in 1983, as well as the duration of exposure on a daily and an annual basis. The actual exposure levels for these workers, however, depends not only upon specifics of formulation, method, and rate of application, but also on the protective measures employed and the operating condition of the equipment used. Exposure estimates do not include accidents, which could greatly increase worker exposure to the pesticide.

Liquid formulations of carbaryl are applied for USDA Forest Service projects. In 1983, in excess of 502,000 acres were treated in three projects at a rate of 1.0 lb a.i./acre (1.12 kg/ha) by aerial application for control of forest insect pests. Of this total acreage, 501,994 acres were treated in one project for control of the western spruce budworm. In addition, 135 acres of nurseries and individual trees were treated by ground application methods at an application rate of 1.0 lbs a.i./acre (1.12 kg/ha) for control of various insect pests and 30 acres of seed orchard were treated at a rate of 2.0 lb.a.i./acre (2.24 kg/ha) for seed orchard insect control (see table 1-2). For these applications, a total of 244 individuals were potentially exposed for 6 to 8 hours/day for 2 to 120 days/year (Gross 1984). Of the 244 individuals potentially exposed, most (201) were involved in aerial application projects.

4.2 Exposure

The primary exposure to carbaryl that results from application for USDA Forest Service projects is for occupational exposures to those individuals applying the pesticide. Estimates of potential exposure to carbaryl are based on aerial as well as ground application. The maximum aerial application rate for carbaryl (see table 1-2) is 1 lb a.i./acre, and for application from the ground, application rates are as high as 2 lb a.i./acre. In both aerial and ground application, carbaryl is applied as a liquid.

Calculations in table 4-2 that are based on aerial application (e.g., wildlife dermal exposures and water concentrations) assume an application rate of 1.0 lb a.i./acre. Those calculations in table 4-2 that are based on ground spraying (e.g., wildlife oral exposures) assume an application rate of 2.0 lb a.i./acre. Other specific assumptions, based upon the toxicological and chemical characteristics of carbaryl and on its behavior in the environment, are discussed in the following sections. Table 4-2 summarizes the estimates for occupational and environmental exposures by means of immersion, dermal, inhalation, and oral routes.

Table 4-1

USDA Forest Service Application Data for Carbaryl

Method of Application	Average Number of People Exposed Per Project Per Application	Total Number of People Exposed Per Application	Duration of Exposure Per Application				Type of Management (Projects)
			Average		Maximum		
			Hrs/Day	Days/Yr	Hrs/Day	Days/Yr	
Aerial	101	201	3	25	6	27	Timber (2) Research (1)
Ground	1	3	5.3	1.3	8	2	Nursery (3)
Ground Foliar	4	40	4.2	18.1	6	120	Seed orchard (1) Recreation (6) Timber (3)

Source: Gross 1984

Table 4-2

Summary of Occupational Dose, Environmental Exposure, and Toxicity for Carbaryl

	Exposure Route[a]		
Occupational Dose[b]	Dermal	Inhalation	Oral
Mixer/Loader Observer Backpack Sprayer	No dose calculated. See text.	No dose calculated. See text.	No dose calculated. See text.
Environmental Exposure			
Fish	0.10 ppm[c]		
Rabbits	0.404 mg/kg	Negligible	2.10 mg/kg
Deer	0.101 mg/kg	Negligible	2.88 mg/kg
Humans	--[d]	--[d]	--[d]
Fish	NA[e]	0.015	0.04 mg/kg (2 days)
Rabbits	NA	0.009	0.035 mg/kg (2 days)
Deer	NA	0.012	0.047 mg/kg (2 days)
Water	NA	0.000	0.007 mg/kg
Berries	NA	0.001	0.000 mg/kg
Mushrooms	NA	0.001	0.000 mg/kg
Toxicity Summary	LD_{50} >4 g/kg in rats.	Slight irritation and weight loss in guinea pigs exposed to 230 and 390 mg/m^3.	LD_{50}'s of 500 to 850, 438, 710, and 248 mg/kg in rats, mice, rabbits, and chickens.
	Most studies, LC_{50} between 1 and 10 ppm for fish.		

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[a] Assumes ground foliar application at 2.0 lb a.i./acre and aerial application at 1.0 lb a.i./acre.

[b] Occupational doses include all routes of exposure.

[c] Fish exposure results from immersion in water containing carbaryl.

[d] Human environmental exposures vial dermal and inhalation routes will be considerably less than occupational doses. Therefore, occupational doses are used as a worst case rather than an estimate of environmental dermal and inhalation exposure. Oral environmental exposures are estimated for six sources.

[e] NA represents nonapplicable data.

4.2.1 Occupational Exposure

Occupational exposure to carbaryl during USDA Forest Service applications would occur principally during (1) mixing and loading of formulations, (2) application of these formulations, and (3) worker re-entry into nursery or seedbeds for weeding, pruning, and transplanting operations. As indicated by the data presented in table 4-1, application exposures are of relatively short duration and are relatively infrequent for most USDA Forest Service projects in which carbaryl formulations are used.

It is difficult to quantify the potential for occupational exposure to carbaryl during worker re-entry operations in forest nurseries and seedbeds, and specific data are not available on exposure to carbaryl as a result of forest application procedures. Data are available, however, for exposure due to agricultural uses of carbaryl. These data are presented below and are particularly useful since many of the occupational activities for which exposures were monitored are similar to activities that could lead to exposure in forest nursery and seedbed applications.

Gold et al. (1982) monitored 38 individuals who were involved in the application of carbaryl in urban settings. These applicators included greenskeepers, city parks and recreation employees, research agronomists, a suburban truck farmer, a suburban orchard grower, and home gardeners. The authors reported that, of the 38 applicators, the mean duration of exposure was 49.2 minutes (range 5 to 235 minutes). Exposures were: mean external exposure (pads attached to outside of the clothing) 3.85 µg/cm^2/hour (range of 0 to 24.5 µg/cm^2/hour); mean internal exposure (pads placed beneath the clothing) 0.26 µg/cm^2/hour (range of 0 to 3.38 µg/cm^2/hour); mean hand exposure of 17.73 µg/cm^2/hour (range of 0.02 to 214.21 µg/cm^2/hour); and mean air concentration of 0.02 µg/l (range of 0 to 0.28 µg/l). The total dermal exposure was reported to be (mean) 0.42 mg/kg body weight/hour (range of 0.01 to 2.86 mg/kg body weight/hour) and the total respiratory exposure (mean) was reported to be 0.79 µg/kg body weight/hour (range 0 to 0.7 µg/kg body weight/hour). Only a small change was reported for serum or erythrocyte cholinesterase activity (-1.01 percent and -1.39 percent, respectively) for applicators.

In a similar study with professional pesticide applicators, the same authors (Leavitt et al. 1982) monitored dermal and respiratory exposure to applicators during application of an 80 percent wettable powder formulati by means of a power sprayer. The mean duration of exposure was 33.3 minutes (range from 17 to 64 minutes). Mean external exposure (pad outside of the clothing) was 15 µg/cm^2/hour (range of 2.7 to 30.2 µg/cm^2/hour); mean internal exposure (pad underneath the clothing) was 1.6 µg/cm^2/hour (range 0 to 11.3 µg/cm^2/hour); the mean hand exposure was 57.2 µg/cm^2/hour (range 0.1 to 180.6 µg/cm^2/hour); and the mean air concentration was

0.1 μg/l (range of 0 to 0.1 μg/l). The total dermal exposure was reported to be (mean) 128.4 mg/hour (range of 30.8 to 401.2 mg/hour) and the total respiratory exposure was reported to be (mean) 0.1 mg/hour (range of 0 to 0.2 mg/hour).

Zwieg et al. (1984) monitored the dermal exposure to carbaryl of 18 strawberry harvesters for a 3-day period on days 15 through 17 following application of carbaryl at a rate of 2 lb a.i./acre (2.24 kg/ha). Mean exposure of the lower legs of the subjects was 0.05 mg/hour and of the hands and arms combined was 1.89 mg/hour.

Wyrobek et al. (1981) reported a study in which semen from 50 men exposed to carbaryl in a production plant for durations of 1 to 18 years was examined for changes in sperm count, morphology, and frequency of sperm carrying double flourescent bodies. They found a significant increase ($p<0.005$) in the proportion of sperm with abnormal head shapes compared to the control group among the workers exposed at the time of sampling, but not among workers that had been away from such exposure (for an average of 6.3 years). The authors of this study were not able to relate the increase in the proportion of these abnormalities to exposure as determined by job category, however.

4.2.2 Environmental Exposure

The potential for environmental exposure to carbaryl formulations as a consequence of USDA Forest Service application is considered in this analysis to be negligible compared to the potential for occupational exposure. Environmental exposures for humans occur when forest visitors or others not directly involved in spray applications come in contact with spray or with sprayed foliage, inhale spray mist, eat plants or animals contaminated with carbaryl or its metabolites, or drink water containing carbaryl or its metabolites. Animals, both terrestrial and aquatic, are subject to both direct and indirect environmental exposures as well.

4.2.2.1 Dermal Exposures. Human dermal environmental exposures would be less than occupational exposures, since only spray operators and observers are directly involved with actual activities on the spray units. A casual visitor to spray units should be expected to receive an exposure much less by orders of magnitude than that of forest applicators.

Animals in the target spray zone, however, are subject to dermal exposure. The MITRE Corporation derived a simple methodology to estimate dermal exposures to small and large mammals. Contamination levels in deer were based on Krynski et al. (1982) and USDA Forest Service (1985), and in rabbits based on Shah and Guthrie (1977) for dermal adsorption. Sizes of animals were estimated from typical measurements of North American mammals (Hall and Kelson, 1959). Rabbits and deer, representing both small and large game animals respectively, have the following estimated dermal exposures based on 1.0 lb a.i./acre aerial spray:

Rabbits in Aerial Spray Zone: 0.404 mg/kg (0.404 mg/kg at 1 lb/acre application rate)

Deer in Aerial Spray Zone: 0.101 mg/kg (0.101 mg/kg at 1 lb/acre application rate)

4.2.2.2 Inhalation Exposures. Human inhalation environmental exposures would be less than occupational exposures since spray operators, involved with activities on the spray units, are more likely to be subject to spray mist than a casual visitor. Thus a casual forest visitor should be expected to receive an inhalation exposure orders of magnitude less than that of a backpack sprayer. Environmental inhalation exposures of animals in the spray target area would occur on a one-time basis and would be limited to a time frame that can be measured in minutes. Inhalation of carbaryl vapor would be negligible since the vapor pressure of carbaryl is low (less than 5×10^{-3} mmHG at 78.8 °F; Kuhr and Dorough 1976). Environmental inhalation exposure is therefore expected to be so small that it has been neglected in this analysis.

4.2.2.3 Immersion Exposures. Aquatic organisms are subject to environmental exposures from immersion in streams and ponds adjacent to target spray zones. The exposure assessment methodology used below assumes that 100 feet downwind deposits would be 1 percent of the deposition rate of pesticide on target. Actual data with aerial application of carbaryl (Currier et al. 1982) indicate that actual deposition of carbaryl approximately 230 feet (76 meters) downwind range from 0.14 to 3.4 percent of the targeted deposition rate. Detectable carbaryl was found as far away as 3.5 km (kilometer).

For the purposes of this analysis, it is assumed that maximum spray drift 100 feet from the target area will result in deposition of 10 percent of the hypothetical deposition in the target zone, or that waterbodies 100 feet from the target spray zone would experience a maximum concentration of 0.10 ppm carbaryl immediately after application at a rate of 1 lb/acre a.i. This maximum concentration would be very quickly diluted by streamflow and reduced by either hydrolysis or photolysis. Assuming a buffer zone of approximately 100 feet between aerial spray zones and waterbodies, environmental exposures for aquatic organisms would be equivalent to the estimated concentration of carbaryl in water, calculated as:

Water concentration: 0.10 ppm (0.10 ppm at 1.0 lb/acre aerial application rate)

Due to the low potential for leaching of carbaryl from soils, only negligible amounts of the pesticide would be expected to be carried into streams and ponds, even during rainfall events soon after application.

4.2.2.4 Oral Exposures. Oral environmental exposures would occur for wildlife eating contaminated vegetation and for humans consuming fish, deer, rabbit water, berries, and mushrooms. A simple exposure model was developed by MITRE, based upon the methods described in Orlova and Zhalbe (1968) and Hayes (1982). Weight estimates and evaluation of foods consumed by wildlife are based upon methods in Giles (1969) and in USDA Forest Service (1985). To estimate oral exposures for deer and rabbits, representing game animals potentially eaten by humans, assume vegetation is sprayed from the ground at the maximum rate for USDA Forest Service projects using carbaryl, which is 2.0 lb a.i./acre, and that rabbit brouse is partially shielded by overstory. These estimates are as follows:

Deer:	2.88 mg/kg (1.44 mg/kg at 1 lb/acre application)
Rabbit:	2.10 mg/kg (1.05 mg/kg at 1 lb/acre application)

Estimates of human oral exposures from consumption of fish, deer, and rabbit require, as a starting point, estimates of maximum tissue concentrations of carbaryl in fish, deer, and rabbit.

Fish tissue concentrations of 0.75 ppm (0.75 mg/kg) are derived as follows:

- Water concentration: 0.100 ppm
- Bioconcentration: 7.5 (actual value reported by Kanazawa (1975) was a maximum of 7.5 ppm after 1 day of exposure in a tank containing 1 ppm carbaryl)

Deer and rabbit tissue concentrations include carbaryl accumulated from both oral and dermal exposure (Krynski et al. 1982, Shah and Guthrie 1977). Tissue concentration estimates for deer and rabbit assume that dermally applied carbaryl is absorbed at a rate of 10 percent, and that 10 percent of the carbaryl ingested by deer and rabbits is assimilated into muscle tissue (U.S. Department of Agriculture). Data presented in appendix H indicate that very little, if any of ingested or dermally applied carbaryl is found in animal muscle tissue, and that the actual bioaccumulation values for dermal and oral exposures in mammals are approximately one order of magnitude less than those used in this analysis. It is also assumed that oral exposure of deer and rabbit to carbaryl lasts only for 2 days due to the moderate to short environmental persistence of carbaryl in the environment. Deer tissue concentrations of carbaryl are derived as follows:

Dermal Exposure contribution assumes 1 percent absorption of a single exposure, or 0.101 mg/kg (dermal exposure) x 0.10 = 0.0101 mg/kg.

Oral Exposure contribution assumes 10 percent assimilation of 2 days feeding, or 2.88 mg/kg/day (oral exposure) x 0.10 x 2 days = 0.576 mg/kg.

Total Deer Tissue concentration = 0.0101 mg/kg + 0.576 mg/kg = 0.5861 mg/kg (0.586 ppm).

Rabbit tissue concentrations of carbaryl are derived in the same way as for deer, as follows:

Dermal Exposure contribution assumes 10 percent absorption of a single exposure, or 0.404 mg/kg (dermal exposure) x 0.10 = 0.0404 mg/kg.

Oral Exposure contribution assumes 10 percent assimilation of 2 days feeding, or 2.10 mg/kg/day (oral exposure) x 0.10 x 2 days = 0.410 mg/kg.

Total Rabbit Tissue concentration = 0.0404 mg/kg + 0.410 mg/kg = 0.4504 mg/kg (0.450 ppm).

Based on the above fish, deer, and rabbit tissue concentrations and human consumption rates, the following estimates of maximum human oral exposures to carbaryl from eating meat are derived as follows:

Fish Meat:	0.75 mg/kg x 2 days x 0.5 kg/day/person divided by 50 kg person = 0.0150 mg/kg
Deer Meat:	0.586 mg/kg x 2 days x 0.5 kg/day/person divided by 50 kg person = 0.0117 mg/kg (0.012 mg/kg)
Rabbit Meat:	0.450 mg/kg x 2 days x 0.5 kg/day/person divided by 50 kg person = 0.0090 mg/kg

Based on assumptions stated in the introduction to the pesticide background statements, at an application rate of 1.0 lb a.i./acre (aerial), water and plant consumption by humans would result in the following oral exposures to carbaryl:

Water: 0.0004 mg/kg (0.0004 mg/kg at 1 lb/acre application)
(0.000 mg/kg)

Berries: 0.001 mg/kg (0.001 mg/kg at 1 lb/acre application)

Mushrooms: 0.0005 mg/kg (0.0005 mg/kg at 1 lb/acre application)
(0.001 mg/kg)

4.3 Hazard Assessment

The toxicological properties of carbaryl and of its metabolite, 1-naphthol, to birds and to small mammals have been summarized in table 2-1 and are detailed in section 2 and the appendices of this pesticide background statement. Although carbaryl has a relatively high acute oral toxicity to mammals by ingestion, humans and wildlife would not be expected to ingest carbaryl at toxic doses (high acute doses) as a result of application by the USDA Forest Service. Table 4-2 compares the data on toxicity of carbaryl in vertebrates by various routes to estimated exposures that could occur as a result of USDA Forest Service application.

The typical mode of entry for carbaryl to wild mammals, for example, during an aerial spray, would be dermal exposure, the toxicity of which is very low. For birds, the acute toxicity is slight to moderate, with moderate toxicity due to chronic exposure. For insect eating birds, a chronic exposure may occur when birds eat the contaminated food supply. However, since birds prefer live prey, they normally would fly offsite to feed in areas where insects and caterpillars were more abundant. In large area sprays, encompassing many overlapping bird territories, as the prey species are diminished, the insectivorous species may be secondarily affected. This could be mitigated by leaving patches of unsprayed areas, where birds would then concentrate to reduce the surviving caterpillars.

For aquatic invertebrates, there is a moderate to high toxicity reported in laboratory studies. In repeated field tests, however, the benthos did not appear to be affected. If prey species (nontarget) are exposed, they could pass the carbaryl on through the aquatic food chain to fish. The bioconcentration factors reported are less than 10, so this hazard would not be significant, except immediately following a spray, since fish do not retain carbaryl. The toxicity of carbaryl to fish in laboratory studies varied from slight to moderate, but in field tests neither adult or fry were negatively affected. Slight decreases in ChE were noted but not enough to harm fish.

Cranmer (1986) produced a definitive review of carbaryl, which included both toxicological review and risk analysis. Any future risk assessment should refer to his evaluation of models and conclusions. Human mortality with carbaryl incidents is a very rare event, including suicide attempts. Forced oral doses (in excess of 200 mg/kg) are required to kill mammals. At sublethal doses (10-100 mg/kg) a profound but rapidly reversible effect on neurological functions has been reported. Doses tested at approximate LD_{50} levels, with obvious toxic responses to pregnant animals, have produced structural malformations in several mammalian species. Risk of developmental toxicity resulting from dietary exposure to humans is likely to be exceedingly small. The lowest NOEL in dogs was 2.0 mg/kg, which represents a 400,000-fold safety factor. Cranmer (1986) concluded carbaryl does not pose an unreasonable risk to humans with regard to teratogenic potential when approved patterns of use are followed.

At high doses toxic to the test animal, carbaryl can produce changes in target genetic apparatus. One concern is the combined reaction product of nitrite and carbaryl, N-nitrosamide, which is highly mutagenic in some _in vitro_ systems. However, no data _in vivo_ supports the contention that carbaryl use poses a hazard to future generations. No oncogenicity has been attributed to carbaryl, but N-nitrosocarbaryl has raised questions of carcinogenicity. Assuming human exposure by consumption of both carbaryl and nitrite, using other likely assumptions and reasonable models, cancer risks are typically in the order of 1×10^{-9} to 1×10^{-12}. The United States agencies usually find 10^{-6} to present an acceptable level of risk.

Carbaryl at doses producing subclinical neurotoxic symptoms has been found to produce a variety of immune system effects that are reversible and non-life-threatening. Carbaryl does not appear to represent a risk factor to the human immune system.

There is a great deal of data available from occupational exposure to carbaryl over 25 years. Epidemiology studies have not shown delayed neurotoxic effects, dysmorphic sperm, or viral enhancement in humans (Cranmer 1986).

Carbaryl does not appear to be teratogenic or to have significant reproductive effects, nor does it seem to be carcinogenic, although some test systems have indicated weak mutagenicity. Based upon the available data, it is anticipated that use of carbaryl by the USDA Forest Service will not result in hazards to humans and wildlife at application rates indicated if proper caution is exercised.

Appendix A

Summary of Carbaryl Toxicity Data for Microorganisms

Appendix A

Summary of Carbaryl Toxicity Data for Microorganisms

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl	Marine phyto-plankton 5 species	Surface absorption, laboratory cultures	Up to 10 days	Growth was totally inhibited by 10 ppm in cultures of *Proloccus* sp., *Chlorella* sp., and *Dunaliella euchlora*; by 1 ppm in cultures of *Monochrysis lutheri*; and by less than 0.1 ppm in *Phacodactylum tricornutum*.	--	Ukeles 1962 in Butler 1977 and in Ware and Roan 1970
Carbaryl (99.7% material)	Planktonic algae cultures	Surface absorption, laboratory culture	2 wks	Population growth of algae in 0.01, 0.1, or 1.0 ppm carbaryl did not differ significantly from controls. Some growth inhibition noted at 10 ppm. At 25 ppm, growth was eliminated in 23% of replicate cultures.	Algae isolated from river water samples.	Butler et al. 1975
Carbaryl	Blue-green algae (*Microcystis aeruginosa*)	Surface absorption, laboratory culture	8 days	Toxicity threshold for inhibition of cell growth = 0.033 ppm.	Tested at pH 7.0, 27 °C, exposed to constant artificial light.	Bringmann and Kuhn 1978
Carbaryl	Green algae (*Scenedesmus quadricaudata*)	Surface absorption, laboratory culture	8 days	Toxicity threshold for inhibition of cell growth = 1.35 ppm.	Tested at pH 7.0, 27 °C, exposed to constant artificial light.	Bringmann and Kuhn 1978

Appendix A (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl	Freshwater plan tonic green algae (Scenedesmus quadricaudata)	Surface absorption, unialgal laboratory culture; static conditions.	6 days	Algae cultured in 0.1 and 1.0 ppm. Growth significantly stimulated at both concs. compared to controls in terms of cell numbers, carbon assimilation, and biomass. By day 6, cell numbers were 23 and 32% greater than controls; carbon assimilation was 17 and 96% greater than controls; and biomass was 144 and 157% greater than controls in cultures with 0.1 and 1.0 ppm carbaryl, respectively.	Cultures maintained at 70 ± 2 oF (21 ± 1 oC).	Stadnyk et al. 1971
Carbaryl	Algae (Scenedesmus quadricaudata)	Surface absorption, laboratory culture	10 days	Carbaryl at all conc. caused a decrease in chlorophyll content compared to controls. Carbaryl concs. of 0.1, 10, 100, and 1,000 ppm resulted in decreases of chlorophyll compared to controls (100%) of 61.8%, 77.2% 43.8%, 42.9%, and 36.1%, respectively.	--	Lejczak 1977

Appendix A (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl	Salt marsh pool algae: *Amphiprora*, *Amphora*, *Nitzschia*, *Chlorococcum*, and *Gonyaulax*	Surface absorption in single-species laboratory cultures	Up to 96 hrs	During first 48 hrs, growth rates of all cultures but Gonyaulax were significantly ($p < 0.1$) inhibited by 1 ppm carbaryl or more. During second 48 hrs, *Amphiprora* cultures were reduced with 10 ppm carbaryl; *Amphora* cultures were reduced with 10 ppm and 1 ppm but not 2 ppm carbaryl; *Nitzschia* cultures were reduced with 2 ppm carbaryl; and *Chlorococcum* cultures were reduced with 10, 2, and 1 ppm carbaryl.	Growth rates compared to controls exposed to acetane solvent.	Maly and Ruber 1983
Sevin	Marine diatom (*Coscinodiscus concinnus*)	Surface absorption, laboratory culture	5 days	Growth rates were 91, 82, 80, 77, 64, and 54% of controls at concs. of 1, 5, 10, 20, 40, and 50 ppb carbaryl, respectively.	Rate of cell division decreased with carbaryl compared to controls.	Ramachandran et al. 1980
Carbaryl (Sevin)	Free-living amoeba; *Acanthamoeba castellanii*	Surface absorption, laboratory culture	6 days	At 10 ppm carbaryl, growth and reproduction of amoeba reduced to 59 ± 8% of control ($p = 0.05$).	Incubated at 30 °C Pesticide dissolved in 10 μl acetone; controls also received 10 μl acetone.	Prescott et al. 1977

Appendix A (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl	Protozoan culture (dominated by *Euplotes* sp.; Ciliata)	Surface absorption, laboratory culture	24 hrs	Mean mortalities of 50% or more were observed at concs. of 1 ppm or more tested). At 6, 8, and 10 ppm mortalities were 90% or more.	--	Weber et al. 1982
Carbaryl	Protozoan (*Paramecium caudatum*; Ciliata)	Surface absorption, laboratory culture	96 hrs	LC_{50}'s of 7.9, 12.4, and 10.2 ppm for 24, 48, and 96 hrs exposure, respectively.	--	Lejczak 1977
Sevin	Bacterium (*Bacillus subtilis*)	Surface absorption, laboratory culture	6 hrs	Growth inhibited to 50% of control at conc. 0.07% Sevin.	--	DeGiovanni-Donnelly et al. 1967
Carbaryl (80% WP)	*Bacillus thuringiensis* (two commercial formulations; Biotrol BTB and Biotrol XK)	Surface absorption, inert surface	Up to 4 wks	Carbaryl at 0.47 g a.i./100 ml had no significant effect on viability of spores Biotrol BTB, but caused significant time-related decrease in spore count for Biotrol XK.	--	Chen et al. 1974
Carbaryl	Filamentous bacterium (*Sphaerotilus natans*)	Surface absorption, laboratory culture	72 hrs	Carbaryl at concs. 1, 10, 100, 1,000, and 10,000 ppm increased dry weight compared to controls (100%) of 290%, 66%, 41%, 184%, and 1,155% respectively.	--	Lejczak 1977

Appendix A (Concluded)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl 50 (50% WP)	Fungus; Trichoderma viride	Surface absorption, laboratory culture	Up to 14 days	Carbaryl at conc. 0.2% (2,000 ppm) did not inhibit germination of spores (0.8% ungerminated) but did inhibit sporulation and caused colony to turn white.	Incubated at 20 to 21 °C.	Baicu 1982
Carbaryl (Mervyn 85% a.i.)	Entomo-pathogenic fungus (Verticillium lecanii)	Surface absorption, laboratory culture	10 days for mycelial growth, overnight for germination.	Germination and mycelial growth were observed in cultures at 150, 1,500, and 15,000 ppm. Germination was not inhibited at 150 ppm or 1,500 ppm; and was not quantified at 15,000 ppm but many germinated cauidia were observed. Mycelial growth was inhibited 29% at 150 ppm; 40% at 1,500 ppm; and 62% at 15,000 ppm.	Mycelial growth measured in terms of colony diam. Cultures maintained at 20 to 21 °C.	Hall 1981
Carbaryl	Entomopathoenic fungus (Lagenidium giganteum). Two isolates (N. Carolina and Louisiana) tested.	Surface absorption, laboratory culture	Up to 7 days	IC_{50}'s (conc. at which 50% inhibition compared to controls) for mycelial growth were 43.7 ppm (35 to 54 ppm) for NC isolate and 39.4 ppm (32 to 49 ppm) for LA isolate. IC_{10}'s were 13.4 and 12.6 ppm for the two isolates.	95% confidence limits in parentheses. Maximum conc. at which zoospores were produced was 5.1 ppm for NC isolate and 12.6 ppm for LA isolate.	Merriam and Axtell 1983

Appendix B

Summary of Carbaryl Toxicity Data for Invertebrates

Apppendix B

Summary of Carbaryl Toxicity Data for Invertebrates

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Sevin (80% WP)	Bent-nosed clam (Macoma nasuta; Pelycopoda)	Surface absorption, static bioassay	96 hrs	Clams were exposed to 15, 20, 25, and 30 ppm. Estimated 48-hr and 96-hr TL_{50}'s were 27.5 and 17.0 ppm, respectively.	Histopathology included necrosis of epithalial tissues (gill, mantle, siphon, and suprabranchial gland).	Armstrong and Millemann 1974a
Carbaryl (80% WP)	Matrid clam (Rangia cuneata; Pelycopoda)	Surface absorption, static bioassay	96 hrs	24, 48, 72, and 96-hr TL_{50}'s were 2,000, 1,860, 350, and 125 ppm, respectively.	—	Chaiyaroch et al. 1975
Sevin	Cockle clam (Clinocardium nuttalli; Pelycopoda)	Surface absorption, static bioassay	9 days	96-hrs median tolerance limit was 3.75 ppm for juveniles. After 3 days exposure, all larvae exposed to 0.8 ppm or greater were dead. At 9 days, larvae exposed to 0.4 ppm had 14% reduction in shell growth compared to controls.	Tested in seawater at 25 pp thousand salinity and 19± 2.0 °C. Metabolite, 1-naphthol, was more toxic than Sevin, with 96-hr median tolerance limit of 2.7 ppm for juveniles.	Butler et al. 1968
Sevin	Bay mussel (Mytilus edulis; Pelycopoda)	Surface absorption	Exposed 1 hr and observed to 48-hr stage.	EC_{50} (anomalous development of 50% of test organisms) was 5.3 and 24.0 ppm for first polar body and 32-hour stage, respectively.	EC_{50} for metabolite; 1-naphthol, was 5.2 ppm for first polar body stage.	Armstrong and Millemann 1974b

Appendix B (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl	Common mussel (*Mytilus edulis*; Pelecypod)	Surface absorption, static bioassay	48 hrs	The EC_{50} for byssal attachment in mussels was >30 ppm at both 24 and 48 hrs.	Temp. 20 ± 2 °C.	Roberts 1975
Carbaryl	Oyster (*Crassostrea virginica*; Pelecypod)	Surface absorption	24 hrs	Growth of young oyster inhibited by 1 ppm.	--	Butler et al. 1962 in Roberts 1975
Carbaryl	Earthworm (*Eisenia foetida*; Oligochaeta)	Surface absorption	Either 30 mins in solution (observed for 80 days) or 30 days in soil mixed with carbaryl.	LC_{50} = 200 to 800 ppm for 30-min exposure to solution. There was no mortality after 14 days in soil with up to 64 ppm carbaryl.	Sublethal effects included toxic tremors and inhibited burying behavior. ChE activity was 87% of control 14 days after exposure to 200 ppm.	Stenersen 1979a and 1979b
Carbaryl	Earthworm (*Allolobophora caliginosa*; Oligochaeta)	Surface absorption	Either 30 mins in solution (observed for 80 days) or 30 days in soil mixed with carbaryl.	LC_{50} = 3.1 to 6.3 ppm for 30 min-exposure to solution. In soil, 4 ppm was lethal to all worms within 2 days.	--	Stenersen 1979a
Carbaryl	Earthworm (*Allolobophora chlorotica*; Oligochaeta)	Surface absorption	Either 30 mins in solution (observed for 80 days) or 30 days in soil mixed with carbaryl.	LC_{50} approximately 200 ppm for 30-min exposure to solution. In soil, 4 ppm lethal to approximately 50% of worms and 8 ppm lethal to all worms within 5 days.	--	Stenersen 1979a

Appendix B (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl	Earthworm (*Lumbricus rubellus*; *Oligochaeta*)	Surface absorption	Either 30 mins in solution (observed for 80 days) or 30 days in soil mixed with carbaryl.	LC_{50} = 25 to 50 ppm for 30 min exposure to solution. In soil, 4 ppm was lethal to all worms within 2 days.	--	Stenersen 1979a
Carbaryl	Earthworm (*Eisenia yoetida*; *Oligochaeta*)	Surface absorption on filter paper.	48 hrs	LC_{50} = 9.0 $\mu g/cm^2$	95% confidence limits 4.3 to 19.0 $\mu g/cm^2$. Initial signs of toxicity included curling and coiling followed by rigidity and contraction. Carbaryl deemed "extremely toxic" to *E. foetida*.	Roberts and Dorough 1984
Carbaryl	Earthworm (*Lumbricus rubellus*; *Oligochaeta*)	Surface absorption on filter paper.	48 hrs	LC_{50} = 0.28 $\mu g/cm^2$	95% confidence limits 0.09 to 0.84 $\mu g/cm^2$.	Roberts and Dorough 1984
Carbaryl (WP)	Earthworm (*Lumbricus terrestris*; *Oligochaeta*)	Surface absorption in field plots.	3 wks	Application at 2 lb a.i./acre resulted in reduced numbers (40.2% of control) and biomass (31.7% of control) of worm populations.	Study conducted in early summer in sandy loam soil pasture land.	Thompson 1971

Appendix B (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Sevin	Earthworm (*Pontoscolex corethrurus*; Oligochaeta)	Surface absorption in soil cultures.	1 mo	Concentrations below 100 ppm increase AChE activity, concs. above 250 ppm inhibit activity.	—	Kale and Krishnamoorthy 1982
Carbaryl	Tubificid worm (*Branchiura sowerbyi*; Oligochaeta)	Surface absorption, static bioassay	72 hrs	Exposure to 4.0 ppm for 72 hrs resulted in 100, 0, and 100% mortality at at 4.4, 21, and 32.2 °C. At 21 °C worms exhibited beaded appearance.	All worms survived 90 days at 4 ppm, temp. stated but presumed to be 21 °C.	Nagvi 1973
Carbaryl (50% WP)	Predatory mite (*Amblyseius fallacis*; Acarina)	Surface absorption-dipped into solution.	48 hrs	Adult females dipped into solution of 119 g/100 l for 5 sec., then observed 48 hrs. Mortalities were 100 and 89% in two strains.	Animals maintained at 25 °C 95% relative humidity.	Hislop and Prokopy 1981
Carbaryl (50% WP)	Predatory mite (*Euseius hibisci*; Acarina)	Surface absorption, exposed to treated leaves.	48 hrs	Mortalities in two strains were reported to be 38.4, 82.2, and 90.3% at 450, 900, and 1,800 mg a.i./l concs., for one strain and 100% for 225, 450, and 900 mg a.i./l for the other strain.	Post-test fecundity of survivors was normal.	Tanigoshi and Cangdon 1983

Appendix B (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl	Predatory mite (*Amblyseius* (*Euseius*) *hibisci*; *Acarina*)	Surface absorption, eggs laid on treated lemon leaves	Not given.	>10 ppm was required to prevent egg hatch; 10 ppm resulted in residues that prevented development of immature mites.	--	Jeppson et al. 1975
Carbaryl (Sevin, technical)	Crawfish (*Procambarus acutus*, *Crustacea*)	Surface absorption, static bioassay	96 hrs	LC_{50} = 0.5 ppm	Average size of crawfish was 0.4 g (juveniles), pH = 8.4, and hardness = 100 mg/l.	Cheah et al. 1980a
Carbaryl (10% granular)	White river crawfish (*Procambarus acutus*; *Crustacea*) (juveniles)	Surface absorption, static bioassay	96 hrs	LC_{50} = 0.5 ppm	95% confidence limits = 0.4 to 0.6 ppm.	Carter and Graves 1973 in Cheah et al. 1978 and 1980b
Carbaryl (41.8% a.i. emulsifiable concentrate)	Red crayfish (*Procambarus clarki*; *Crustacea*)	Surface absorption, static bioassay	36 hrs	LC_{50} = 0.74 ppm (equivalent to 1.0 lb/1/2 acre-ft.)	Water temp. of 22.2 to 25.5 °C; 10 to 25 g animals (95% confidence limits = 0.85 to 1.18 lb/1/2 acre-ft.).	Chang and Lange 1967
Carbaryl (80% WP)	Crayfish (*Procambarus simulans*; *Crustacea*)	Surface absorption, static bioassay	96 hrs	24, 48, 72, and 96-hr TL_{50}'s were 2.87, 2.66, 2.43, and 2.43 ppm.	--	Chaiyarach et al. 1975

Appendix B (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl (technical, 99.5%)	Crayfish (*Procambarus* sp.; Crustacea)	Surface absorption, static bioassay	96 hrs	LC_{50} = 1.90 ppm (1.16 to 3.11 ppm).	95% confidence limits in parentheses. Early instar individuals, 12 °C test temp.	Johnson and Finley 1980
Carbaryl (80% WP)	Grass shrimp (*Palaemonetes kadiakensis*; Crustacea)	Surface absorption, static bioassay	96 hrs	24, 48, 72, and 96-hr TL_{50}'s were 0.41, 0.24, 0.14, and 0.12 ppm.	--	Chaiyarach et al. 1975
Carbaryl (Sevin; 98% a.i.)	Grass shrimp (*Palaemonetes pugio*; Crustacea)	Surface absorption, flow-through avoidance chamber	1 hr	Shrimp exposed 0.1, 0.01, 0.001, or 0.0001 ppm did not exhibit any avoidance reaction to carbaryl during a 1-hr observation.	A 24-hr LC_{50} of 0.038 ppm was cited.	Hansen et al. 1973
Carbaryl (technical, 99.5%)	Seed shrimp (*Cypridopsis vidua*; Crustacea)	Surface absorption, static bioassay	48 hrs	EC_{50} = 0.115 ppm (0.074 to 0.179 ppm).	95% confidence limits in parentheses. Mature individuals, 21 °C test temp.	Johnson and Finley 1980
Carbaryl (technical, 99.5%)	Glass shrimp *Palaeomonetes kadiakensis*; Crustacea)	Surface absorption, static bioassay	96 hrs	LC_{50} = 0.0056 ppm (0.0036 to 0.0083 ppm).	95% confidence limits in parentheses. Mature individuals, 21 °C test temp.	Johnson and Finley 1980
Sevin	Mysid shrimp (*Mysidopsis bahia*, Crustacea)	Surface absorption, flow-through bioassay	96 hrs	LC_{50} > 0.0077 ppm	--	Nimmo et al. 1981

Appendix B (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Sevin	Fiddler crab (*Uca pugilator*; Crustacea)	Surface absorption, static bioassay	--	0.1 ppm lethal to crabs	--	Weis and Mantel 1976
Carbaryl (Sevin)	Dungeness crab (*Cancer magister*; Crustacea)	Surface absorption	--	1 ppm carbaryl did not affect egg hatching in sea water, but inhibited molting of prezoeas to zoeae. Molting delayed by concs. as low as 0.1 ppb.	Early larvae more sensitive than juveniles or adults.	Buchanan et al. 1970 in Hart and Fuller 1979
Carbaryl	Cladoceran (*Daphnia magna*; Crustacea)	Surface absorption, static bioassay	96 hrs	24, 48, 72, and 96-hr LC_{50}'s were 22.90, 16.76, 9.36, and 3.28 ppm.	Tested in filtered river water at 20 °C.	Lejczak 1977
Carbaryl (technical, 99.5%)	Cladocera (*Simocephalus serrulatus*; Crustacea)	Surface absorption, static bioassay	48 hrs	EC_{50} = 0.0076 ppm (0.0062 to 0.0093 ppm)	95% confidence limits in parentheses. First instar, 16 °C test temp.	Johnson and Finley 1980
Carbaryl (technical, 99.5%)	Cladocera (*Daphnia pulex*; Crustacea)	Surface absorption, static bioassay	48 hrs	EC_{50} = 0.0064 ppm (0.0045 to 0.0089 ppm)	95% confidence limits in parentheses. First instar, 16 °C test temp.	Johnson and Finley 1980
Carbaryl	Cladoceran (*Daphnia magna*; Crustacea)	Surface absorption	24 and 48 hrs	24-hr TL_{50} = 0.100 ppm; 48-hr. TL_{50} = 0.035 ppm.	Tested at pH 7.8 to 8.2 and 29 to 32 °C.	Santharam et al. 1976

Appendix B (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl (98% a.i.)	Cladoceran (*Daphnia pulex*; Crustacea)	Surface absorption, static bioassay	18 hrs	LD_{50} = 0.00505 µg in stressed bioassay with 0.4% methanol; 0.008 µg stressed with 2% methanol.	Tested at 23 ± 1 °C.	Bowman et al. 1981 and 1982
Carbaryl (technical, 99.5%)	Scud (*Gammarus lacustris*; Crustacea)	Surface absorption, static bioassay	96 hrs	LC_{50} = 0.022 ppm (0.016 to 0.030 ppm)	95% confidence limits in parentheses. Mature individuals 21 °C test temp.	Johnson and Finley 1980
Carbaryl (technical, 99.5%)	Scud (*Gammarus fasciatus*; Crustacea)	Surface absorption, static bioassay	96 hrs	LC_{50} = 0.026 ppm (0.016 to 0.039 ppm)	95% confidence limits in parentheses. Mature individuals, 21 °C test temp.	Johnson and Finley 1980
Carbaryl (98% a.i.)	Scud (*Hyalella azteca*; Crustacea)	Surface absorption, static bioassay	18 hrs	LD_{50} = 0.0911 µg in stressed bioassay with 0.5% methanol; 0.016 µg stressed with 2% methanol.	Tested at 23 ± 1 °C.	Bowman et al. 1981 and 1982
Carbaryl (technical, 99.5%)	Sowbug (Ascellus brevicaudus; Crustacea)	Surface absorption, static bioassay	96 hrs	LC_{50} = 0.28 (0.214 to 0.367) ppm.	95% confidence limits in parentheses. Mature individuals, 18 °C test temp.	Johnson and Finley 1980
Carbaryl	Ostracod (*Cypretta kawatai*; Crustacea)	Surface absorption, static bioassay	24 and 72 hrs	24-hr EC_{50} = 4.45 (2.93 to 6.75) ppm; 24-hr LC_{50} = 5.28 (3.52 to 7.92) ppm; 72-hr EC_{50} = 1.80 (1.26 to 2.57) ppm; 72-hr LC_{50} = 1.80 (1.27 to 2.55) ppm.	95% confidence limits in parentheses. Exposed in soft water (40 to 48 ppm) at 20 ± 0.5 °C.	Hansen and Kawatski 1976

Appendix B (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl	Honey bee (Apis millifera; Insecta)	Oral in sugar solution	Observed for 24 hrs.	0.4 μg/bee caused 100% mortality, 0.2 μg/bee caused 50% mortality.	--	Argauer et al. 1972
Carbaryl	Honey bee (Apis mellifera; Insecta)	Oral	Observed for 72 hrs while feeding on contaminated pollen.	Mortalities were 8, 36, 38, and 39% among bees fed contaminated honey compared to 5 and 7% among controls.	--	Johansen and Brown 1972
Carbaryl (5% dust)	Honey bee (Apis mellifera; Insecta)	Oral in diet.	Various; up to 11 wks.	Pollen was contaminated with carbaryl to achieve concs. of either 10 or 100 ppm and fed to bees fresh or after weathering. Freshly contaminated pollen resulted in 7,735 and 1,063 dead bees at 100 and 10 ppm, respectively, compared to 157 dead bees fed control pollen (no carbaryl).	Weathered pollen retained its toxic effect, with pollen originally containing 100 ppm still toxic after 2 mos.	Moffett et al. 1970
Carbaryl	Honey bee (Apis mellifera; Insecta)	Oral	48 hrs	Ingestion of 1.0, 0.5, and 0.25 μg/bee resulted in 100, 70, and 70% mortalities.	--	Redfern and Knox 1974

Appendix B (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl (technical; at least 95% pure)	Honey bee (Apis mellifera; Insecta)	Oral	At least 1 day.	LD_{50} = 0.14 μg/bee	Acetone solution in 20% sucrose in water; 0.2 ml fed to groups of 10 bees.	Stevenson et al. 1977
Carbaryl (Sevin® 80 sprayable, Sevin® SL, Sevin® XLR, Sevin-4-oil®, and Sevimol 4®)	Honey bee (Apis mellifera; Insecta	Surface absorption, topical application	Observed for 96 hrs after application.	LD_{50}'s in μg/bee were 1.540, 3.602, 9.360, 13.721, 26.531 for Sevin 80 Sprayable, 4-oil, Sevimol 4, SL, and XLR, respectively.	In field tests, Sevin XLR was 4.5-fold and Sevin SL was 2-fold less toxic bees than was Sevin 80 Sprayable.	Atkins 1981
Carbaryl (50% WP)	Honey bee (Apis cerana indica; Insecta)	Surface absorption, topical application	Observed 6 hrs after application.	Bees sprayed with 0.31% solution had 85% mortality at 30 mins., 100% mortality at 1 hr after spray.	AChE inhibition was 40% for hyperactive bees, 56% for bees during knockdown, and 91% for bees with acute paralysis.	Bai and Reddy 1977a
Carbaryl (50% WP)	Indian Honey bee (Apis cerana indica; Insecta)	Surface absorption, topical application	Up to 72 hrs	Bees sprayed with 1 ml of 0.31% solution, dried for 5 mins., and then transferred to observation cages. Mortality was 85% at 30 mins. and 100% at 1 hr.	Kept at 27 °C and 40 to 50% relative humidity. Carbaryl was deemed "very highly toxic."	Bai and Reddy 1977b
Carbaryl	Honey bee (Apis mellifera; Insecta)	Surface absorption, topical application to thorax	Observed for 24 hrs.	LD_{50} = 0.1 to 0.2 μg/bee dissolved in 2 μl acetone.	--	Barker 1970

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Appendix B (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl	Honey bee (*Apis mellifera*; Insecta)	Surface absorption, topical application	24 hrs	LD_{50} = 0.96 μg/bee applied to abdomen 0.78 μg/bee to thorax.	Bees caged in groups of 15 at 60 °F after treatment.	Graves and Mackensen 1965
Sevin	Carniolan Honey bee (*Apis* sp.; Insecta)	Surface absorption, topical application	6 hrs	Bees were exposed to glass plates sprayed with Sevin at rates of 1.25 kg/feddan (0.168%); 1.50 kg/feddan (0.200%) or 1.75 kg/feddan (0.235%) and allowed to weather 3, 5, 7, or 10 days before bee exposure. Six-hr mortalities were 100% for 3-day weathered material at 1.50 kg/feddan; and 10-day weathered material at 1.25 kg/feddan (1 feddan = 1.038 acres, Egypt).	--	Ibrahim et al. 1971
Carbaryl	Honey bee (*Apis mellifera*; Insecta)	Topical	48 hrs	Topical application of 10, 1.0, 0.5, and 0.25 μg/bee resulted in 100, 80, 80, and 60% mortalities, respectively.	--	Redfern and Knox 1974
Carbaryl (Sevin 50 WP)	Honey bee (*Apis cerana indica*; Insecta)	Surface absorption, topical application	24 hrs	LC_{50} = 0.02966% (296.6 ppm); 95% confidence limits 382.4 to 229.9 ppm.	Workers sprayed with 2 ml/jar (15 x 10 cm) containing 15 to 18 individuals.	Singh et al. 1974

Appendix B (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl (technical; at least 95% pure)	Honey bee (*Apis mellifera*; Insecta)	Surface absorption, topical application	At least 1 day.	LD_{50} = 1.3 μg/bee	1-μl drop dissolved in acetone applied to anaesthetized worker bee.	Stevenson et al. 1977.
Carbaryl	Honey bee (Apis *mellifera*; Insecta)	Poisoning in the field due to carbaryl application.	--	Carbaryl detected in 28 of 43 samples of bees. 9% of colonies contaminated only with carbaryl did not survive.	Approximately 68% of poisonings in May and early June during spraying for gypsy moth control.	Anderson and Glowa 1984
Carbaryl (80% WP)	Alfalfa Leafcutter bee (*Megachile rotundata*; Insecta)	Surface absorption, treated leaves.	Observed for 24 hrs.	24-hr mortalities were 98, 100, and 82% for bees placed on leaves with residues weathered for 3 hrs, 8 hrs, and 2 days. respectively.	Application rate equivalent to 1 lb/acre.	Johansen 1972
Carbaryl (80% WP)	Alkali bee (*Nomia melanderi*; Insecta)	Surface absorption, treated leaves.	Observed for 24 hrs.	24-hr mortalities were 100, 97, and 78% for bees placed on leaves with residues weathered for 3 hrs, 8 hrs, and 2 days.	Application rate equivalent to 1 lb/acre.	Johansen 1972

Appendix B (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl (80% WP)	Honey bee (*Apis mellifera*; Insecta)	Surface absorption, treated leaves.	Observed for 24 hrs.	24-hr mortalities were 93, 85, and 69% for bees placed on leaves with residues weathered for 3 hrs, 8 hrs, and 2 days.	Application rate equivalent to 1 lb/acre.	Johansen 1972
Carbaryl (80% WP)	Bumble bee (*Bombus centralis*; Insecta)	Surface absorption, treated leaves.	Observed for 24 hrs.	24-hr mortality was 89% for bees placed on leaves with residues weathered for 3 hrs.	Application rate equivalent to 1 lb/acre.	Johansen 1972
Carbaryl (50% WP)	Honey bee (*Apis mellifera*; Insecta)	Surface absorption.	2 wks	A 1-oz. application of 1% suspension to each of 4 groups of ventilation saw cuts in the hive frame resulted in 15% mortality within 6 hrs, indicating moderate toxicity.	Bee activity increased for approximately 2 hrs after application.	Keener and Pless 1974
Carbaryl	Silkworm (*Bombyx mori*; Insecta)	Treated leaves fed to 5th instar larvae.	Not given.	Larvae fed 0.004 and 0.008% had reduced emergence (86 and 42% compared to 96% for controls) laid fewer eggs, laid more abnormal and nonfertilized eggs, had more embryonic deaths, and had fewer eggs hatch than controls.	No statistical analysis provided.	Kuribayshi 1981

Appendix B (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Sevin	Mosquito (*Aedes aegypti*; Insecta	Surface absorption distilled water.	24 hrs	Concentration of 1.0 ppm resulted in 70% mortality larvae. A range of 0.50 to 2.00 ppm resulted in 20 to 80% mortality.	Temperature 28± 1 °C.	Lichtenstein et al. 1966
Carbaryl (98% a.i.)	Container mosquito (*Culex restuans*; Insecta)	Surface absorption, static bioassay	18 hrs	LD_{50} = 11.1 g in a stressed bioassay with 0.5% methanol.	Tested at 23± 1 °C.	Bowman et al. 1981 and 1982
Carbaryl (technical 99.5%)	Stonefly (*Pteronarcella badia*; Insecta)	Surface absorption, static bioassay	96 hrs	LC_{50} = 0.0017 (0.0014 to 0.0024) ppm.	95% confidence limits in parentheses. Niads, 16 °C test temp.	Johnson and Finley 1980
Carbaryl	Stonefly (*Pteronarcys californica*; Insecta)	Surface absorption, static bioassay	96 hrs	LC_{50} = 0.0048 (0.0030 to 0.0077) ppm.	95% confidence limits in parentheses. Second-year class, 16 °C test temp.	Johnson and Finley 1980
Carbaryl (technical, 99.5%)	Stonefly (*Claassenia sabulosa*; Insecta)	Surface absorption, static bioassay	96 hrs	LC_{50} = 0.0056 (0.0039 to 0.0081) ppm.	95% confidence limits in parentheses. Second year class, 16 °C test temp.	Johnson and Finley 1980
Carbaryl (technical, 99.5%)	Stonefly (*Skwala* sp.; Insecta)	Surface absorption, static bioassay	96 hrs	LC_{50} = 0.0036 (0.0024 to 0.0055) ppm.	95% confidence limits in parentheses. Niads, 12 °C test temp.	Johnson and Finley 1980

Appendix B (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl (oil dispersion; 49%)	Stonefly (*Skwala* sp.; Insecta)	Surface absorption, static bioassay	96 hrs	LC_{50} = 0.0092 (0.0074 to 0.0120) ppm.	95% confidence limits in parentheses. First year class, 7 °C test temp.	Johnson and Finley 1980
Carbaryl	Midge (*Chironamus tentans*; Insecta)	Surface absorption, static bioassay	24 and 72 hrs	24-hr EC_{50} = >18.0 ppm; 24-LC_{50} >18.0 ppm 72-hr EC_{50} = 12.0 (10.8 to 13.4) ppm; 72-hr LC_{50} = 5.90 (4.75 to 7.38) ppm.	95% confidence limits in parentheses. Exposed in soft water (40 to 48 ppm) at 20 °C 0.5 °C.	Hansen and Kawatski 1976
Carbaryl (Ortho-Sevin liquid, 27% a.i.)	Aquatic insects (*Hydropsyche* sp. and *Cheumatopsyche* sp.; Insecta)	Surface absorption, static bioassay	24 hrs	Larvae exposed to 0.22, 0.54, and 0.864 ppm all died within 16 hrs. Survival of controls was 97.2% at 24 hrs.	Larvae maintained at 25 to 27 °C, pH 7.3 to 7.9.	Bradt and Williams 1980
Carbaryl	Backswimmer (*Notonecta undulata*; Insecta)	Surface absorption, static bioassay	96 hrs	48-hr LC_{50} = 0.36 ppm; 72-LC_{50} = 0.23 ppm; and 96-hr LC_{50} = 0.20 ppm.	Control mortality averaged 4%. LC_{50} values were uncorrected for control mortality; 25 ± 2°C; field-collected animals.	Federle and Collins 1976
Carbaryl	Aquatic insect (*Eretes sticticus*; Insecta)	Surface absorption, static bioassay	48 hrs	24-hr TL_{50} = 0.91 ppm; 48-hr TL_{50} = 0.89 ppm.	At 1.0 ppm, mortality was 100% within 24 hrs; at 0.8 ppm, mortalities were 20% at 24 hrs and 30% at 48 hrs. There were no mortalities within 48 hrs among animals exposed to 0.6 ppm. Temp. 25° to 32 °C, pH 7.4.	Jeyasingam et al. 1978

Appendix B (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl (10% WDP)	Dragonfly (*Brachythermis contaminata*; Insecta)	Surface absorption, static bioassay	96 hrs	24-hr LC_{50} = 0.1449×10^{-4} ppm; 48-hr LC_{50} 0.1065×10^{-4} ppm; 72-hr LC_{50} = 0.8375×10^{-6} ppm; and 96-hr LC_{50} = 0.6933×10^{-6} ppm.	Nymphs tested at pH 6.8 to 7.2; alkalinity of 28 to 35 ppm $CACO_3$; and temp. 24 ± 3 °C. A "safe harmless" conc. of 0.01729×10^{-4} ppm was calculated. This paper defines ppm as "part per millititre."	Shukla and Mishra 1980
Carbaryl (50% WP)	Aquatic insect (*Ranatra elongata*; Insecta)	Surface absorption, static bioassay	96 hrs	LC_{50} = 0.624 (0.5235 to 0.7440) ppm. LC_{25} = 0.4029 ppm; LC_{75} - 0.9665 ppm.	95% confidence limits for LC_{50} in parentheses. Animals tested at 25 ± 1 °C; pH 7.4 ± 0.2; dissolved oxygen of 7.2 ± 0.3 ppm; and hardness 110.35 ± 1.2 ppm.	Shukla et al. 1982
Carbaryl	Crawling water beetle (*Peltodytes* sp.; Insecta)	Surface absorption, static bioassay	96 hrs	24-hr LC_{50} 100 ppm; 48-hr LC_{50} = 25.0 ppm; 72-hr LC_{50} 6.0 ppm; and 96-hr LC_{50} 3.3 ppm.	Control mortality averaged 5%, LC_{50} values were uncorrected for control mortality; 25 ± 2 °C; field-collected animals.	Federle and Collins 1976
Carbaryl (85% WP)	Green lacewing (*Chrysopa boninensis*; Insecta)	Surface absorption on dry, treated paper.	24 hrs	LC_{50} = 4.39% a.i. (3.162 to 5.168%).	95% confidence limits in parentheses. Third instar larvae tested.	Brettell 1979
Carbaryl	Green lacewing (*Chrysopa carnea*;	Surface absorption	72 hrs	LC_{50} = 104 μg/vial; LC_{90} = 1,894 μg/vial.	Tested in larvae. Insecticide film on inner surface of glass vial.	Plapp and Bull 1978

Appendix B (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Sevin (80% WP)	Brown lacewing (Sympherobius barberi; Insecta)	Surface absorption, topical application to thorax and abdomen.	24 and 48 hrs	1 µl of material (5.6 lb a.i./250 gal. or 2.68 g/l) gave 90% mortalities after 24 and 48 hrs.	--	Reeve and French 1978
Carbaryl (Karbatox 75)	Cricket (Acheta domesticus; Insecta)	Surface absorption among eggs.	From 48 hrs after laying through 26 days (hatching).	Application of 0.1% resulted in 100% egg mortality within 2 days. Application of 0.01% resulted in 10% immediate mortality at hatch. Application of 0.005% and 0.001% resulted in 20% mortality. At lower conc. 0.0005% or less, no differences were observed compared to controls.	Dead embryos treated with 0.01% carbaryl showed abnormal bursting of chorion. Some survivors had abnormal limb development.	Jordan et al. 1979
Carbaryl	Large Milkweed bug (Oncopeltus fasciatus; Insecta)	Topical application to metathorax.	Observed for 24 hrs.	5.0 µg/bug caused 100% mortality, 0.1 µg/bug caused 40% mortality.	--	Argauer et al. 1972
Carbaryl	Western bigeyed bug (Geocoris pallens; Insecta)	Surface absorption	24 hrs	LD_{50} = 1,790 (1,449.8 to 2,248.5) µg/ha on leaves; 3,923.8 µg/ha (2,385.6 to 6,519.8) µg/ha filter paper.	Field-collected animals exposed to residues on cotton leaves or filter paper. 95% confidence limits in parentheses.	Yokoyama et al. 1984

Appendix B (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl	Striped earwig (Labidura riparia; Insecta)	Surface absorption, treated soil.	2 days	Application equivalent to a rate of 1 lb a.i./acre resulted in 38% mortality.	--	Workman 1977
Carbaryl	Ladybird beetle (Menochilus sexmaculatus; Insecta)	Surface absorption, spray applied to larvae.	72 hrs	1 ml of 0.1% material sprayed over petri dishes containing 20 3rd instar larvae resulted in 66.66, 78.06, 89.16, and 97.61% mortality after 12, 24, 48, and 72 hrs, exposure	--	Makar and Jadhav 1981
Carbaryl (50% liquid)	Predatory beetle (Stethorus nigripes; Insecta)	Surface absorption, dipped in solution.	Exposed for 15 sec. and observed up to 18 hrs.	Concentrations of 1.135 l 1,000 l (0.11%) resulted in 100 to 97% mortality within 1 hr and 100% mortality after 18 hrs.	--	Edwards and Hodgson 1973
Carbaryl	3 species of predatory beetle (Stethorus loxtoni, S. nigripes, and S. vagans; Insecta)	Surface absorption, treated filter paper.	Exposed up to 48 hrs.	At 0.1%, there was 100% mortality after 24 hrs for larvae of all 3 species and 100% mortality after 48 hrs for adults of all 3 species.	--	Walters 1976

Appendix B (Concluded)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl (Cont'd)				Mortality of eggs was 40, 20, and 10% for *S. loxani*; *S. nigripes*; and *S. vagans*.		
Carbaryl (50% WP)	Predatory insect (*Reduviolus americoferus*; Insecta)	Dipped in solution for 2 secs.	Observed for 24 hrs.	LC_{50} = 173.91 (90.91 to 255.67) ppm.	95% confidence limits in parentheses.	Martinez and Pienkowski 1983

Appendix C

Summary of Carbaryl Toxicity Data for Fish

Appendix C

Summary of Carbaryl Toxicity Data for Fish

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Sevin (50% WP)	Freshwater carp (*Labeo rohita*)	Surface absorption, static bioassay	30 days	96-hr TL_{50} = 1.87 ppm. Estimated 30-day MATC (maximum acceptable toxicant conc.) = 0.052 to 0.078 ppm.	Tested larval individuals at 24.3 to 28.4 °C; pH 6.8 to 7.6; total hardness 38 to 47 ppm.	Bansal et al. 1980
Sevin (50% WP)	Freshwater carp (*Cirrhina mrigala*)	Surface absorption, static bioassay	30 days	96-hr TL_{50} = 1.73 ppm. Estimated 30-day MATC (mxaimum acceptable toxicant conc.) = 0.052 to 0.078 ppm.	Tested larval individuals at 24.3 to 28.4 °C; pH 6.8 to 7.6; total hardness 38 to 47 ppm.	Bansal et al. 1980
Sevin (50% WP)	Freshwater carp *Cyprinus carpio*)	Surface absorption, static bioassay	30 days	96-hr TL_{50} = 2.0 ppm. Estimated 30-day MATC (maximum acceptable toxicant conc.) = 0.052 to 0.078 ppm.	Tested larval individuals at 24.3 to 28.4 °C; pH 6.8 to 7.6; total hardness 38 to 47 ppm.	Bansal et al. 1980
Sevin (50% WP)	Freshwater carp (*Catla catla*)	Surface absorption, static bioassay	30 days	96-hr TL_{50} = 1.42 ppm. Estimated 30-day MATC (maximum acceptable toxicant conc.) = 0.052 to 0.078 ppm.	Tested larval individual at 24.3 to 28.4 °C; pH 6.8 to 7.6; total hardness 38 to 47 ppm.	Bansal et al. 1980

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl (technical, 99.5%)	Coho salmon	Surface absorption, static bioassay	96 hrs	LC_{50} = 4.34 (3.31 to 5.69) ppm.	95% confidence limits in parentheses. Coho salmon tested at 13 °C, other salmonids tested at 12 °C. Perch and crappie tested at 18 °C. Little alteration was observed in toxicity to cutthroat trout when temp. were raised from 7 to 17 °C but 4- to 11-fold increase in toxicity was observed by similar temp. increase in brook trout and yellow perch. Alkaline test solutions (pH 8.5 to 9.0) were 1.4 to 11.4 times more toxic to trout, salmon, and yellow perch than solutions with lower (6.5 to 7.5) pH. Variation in hardness (12 to 300 ppm) did not affect toxicity to trout or yellow perch. Fish weighed 0.5 to 1.7 g.	Johnson and Finley 1980
	Cutthroat trout			LC_{50} = 7.10 (5.24 to 9.62) ppm.		
	Rainbow trout			LC_{50} = 1.95 (1.45 to 2.63) ppm.		
	Atlantic salmon			LC_{50} = 4.50 (3.82 to 5.31) ppm.		
	Brown trout			LC_{50} = 6.30 (5.52 to 7.19) ppm.		
	Brook trout			LC_{50} = 2.10 (1.68 to 2.62) ppm.		
	Lake trout			LC_{50} 0.69 (0.52 to 0.91) ppm.		
	Black crappie			LC_{50} = [illegible].60 (1.18 to 5.70) ppm.		
	Yellow perch			LC_{50} = 5.10 (4.52 to 5.76) ppm.		

Appendix C (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl (oil dispersion, 49%)	Brook trout	Surface absorption, static bioassay	96 hrs	LC_{50} = 4.50 (3.948 to 5.066) ppm.	95% confidence limits in parenthesis. Trout tested at 12 °C; bluegill at 17 °C. Trout weighed 1.3 g; bluegill weighed 0.7 g.	Johnson and Finley 1980
	Bluegill			LC_{50} = 39.0 (29.732 to 51.157) ppm.		
Carbaryl (technical, 99.5%)	Chinook salmon	Surface absorption, flow-through assay	96 hrs	LC_{50} = 2.40 (1.62 to 3.55) ppm.	95% confidence limits in parentheses. Fingerlings, tested at 12 °C.	Johnson and Finley 1980
Carbaryl (technical, 99%)	Bullhead (Ictaluridae)	Surface absorption, static bioassay	96 hrs	LC_{50} = 20.0 (13.9 to 18.0) ppm.	95% confidence limites in parentheses. Salmonids were tested 13± 0.5 °C, other species were tested at 18 ± 0.5 °C. Fish weighed 0.6 to 1.7 grams at testing; fish of any given species were from the same source and were ±20% of the same weight.	Macek and McAllister 1970
	Catfish (Ictaluridae)			LC_{50} = 15.8 (13.9 to 18.0) ppm.		
	Minnow (Cyprinidae)			LC_{50} = 14.6 (11.7 to 19.8) ppm.		
	Goldfish (Cyprinidae)			LC_{50} = 13.2 (8.31 to 20.8) ppm.		
	Sunfish (Centrarchidae)			LC_{50} = 11.2 (8.14 to 15.5) ppm.		
	Bluegill (Centrarchidae)			LC_{50} = 6.76 (5.22 to 8.76) ppm.		
	Bass (Centrarchidae)			LC_{50} = 6.4 (4.40 to 9.20) ppm.		
	Carp (Cyprinidae)			LC_{50} = 5.28 (4.62 to 6.05) ppm.		
	Rainbow trout			LC_{50} = 4.34 (3.31 to 5.69) ppm.		

Appendix C (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl (technical, 99%) (Cont'd)	Brown trout (Salmonidae)			LC_{50} = 1.95 (1.45 to 2.63) ppm.		
	Perch (Percidae)			LC_{50} = 0.745 (0.611 to 0.904) ppm.		
	Coho Salmon (Salmonidae)			LC_{50} = 0.764 (0.649 to 0.902) ppm.		
Carbaryl (98% a.i.)	Brook trout	Surface absorption, static bioassay	96 hrs	For 1.15 g fish, the 24-hr TL_{50} = 1.83 (1.441 to 2.324) ppm; 48-hr TL_{50} = (1.176 to 1.913) ppm; 72-hr TL_{50} = 1.15 (0.927 to 1.426) ppm; and 96-hr TL_{50} = 1.070 (0.905 to 1.263) ppm. For 2.04 g fish, the 72-hr TL_{50} = 1.64 (1.247 to 2.157) ppm and the 96-hr TL_{50} = 1.45 (1.047 to 2.008) ppm.	95% confidence limits in parentheses. Temp. 12.9 °C, pH 7.2 to 7.6. Tolerance of larger fish was greater than smaller fish.	Post and Schroeder 1971

Appendix C (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl (98% a.i.)	Cutthroat trout	Surface absorption, static bioassay	96 hrs	For 0.37 g fish, the 72-hr TL_{50} = 2.00 (1.399 to 2.860) ppm and the 96-hr TL_{50} = 1.50 (1.176 to 1.913) ppm. For 1.30-g fish, the 96-hr TL_{50} = 2.169 (2.067 to 2.276) ppm.	95% confidence limits in parentheses. Temp. 12.9 °C, pH 7.2 to 7.6. Tolerance of larger fish was greater than smaller fish.	Post and Schroeder 1971
Carbaryl (98% a.i.)	Rainbow trout	Surface absorption, static bioassay	96 hrs	96-hr TL_{50} = 1.47 (0.980 to 2.205) ppm for 1.24 g fish.	95% confidence limits in parentheses. Temp. 12.9 °C pH 7.2 to 7.6	Post and Schroeder 1971
Carbaryl (98% a.i.)	Coho Salmon	Surface absorption, static bioassay	96 hrs	24-hr TL_{50} = 2.95 (2.201 to 3.953) ppm; 48-hr TL_{50} = 2.70 (1.929 to 3.789) ppm; 72-hr TL_{50} = 1.69 (1.341 to 2.129) ppm and 96-hr TL_{50} = 1.30 (1.074 to 1.573) ppm for 1.5 g fish.	95% confidence limits in parentheses. Temp. 12.9 °C, pH 7.2 to 7.6.	Post and Schroeder 1971
Carbaryl (technical, 99.5%)	Rainbow trout	Surface absorption, static bioassay	96 hrs	LC_{50} = 2.2 (1.7 to 2.8) ppm.	95% confidence limits in parentheses. Tested at 17 °C, pH of 7.4, hardness of 40 ppm. Toxicity was not appreciably affects by differences in temp., pH, or hardness.	Sanders et al. 1983

Appendix C (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl (technical, 99.5%)	Bluegill	Surface absorption, static bioassay	96 hrs	LC_{50} = 7.0 (5.1 to 9.8) ppm.	95% confidence limits in parentheses. Tested at 22 °C, pH of 7.4, hardness of 40 ppm. Toxicity to bluegills increased 3-fold (5.4 ppm to 1.8 ppm) when pH was changed from 6.5 to 8.5.	Sanders et al. 1983
	Fathead minnow			LC_{50} = 14.6 (11.7 to 19.8) ppm.		
	Channel catfish			LC_{50} = 15.8 (13.9 to 18.0) ppm.		
Carbaryl (technical, 99.5%)	Brook trout	Surface absorption, static bioassay	96 hrs	LC_{50} = 3.0 (2.0 to 4.5) ppm at 7 °C, 40 ppm hardness, pH 7.5.	95% confidence limits are in parentheses. Toxicity to brook trout increased with increased temp., increased hardness, and increased pH. As part of study, brook trout with body residues of 0.08 ppb PCB (Aroclor 254) reported to have 96-hr LC_{50} of 5.9 (5.1 to 6.9) ppm, with residues of 0.4 ppb PCB to have 96-hr LC_{50} of 5.0 (4.1 to 6.0) ppm, and with 2.5 ppb PCB to have 96-hr LC_{50} of 2.5 (1.7 to 3.7) ppm.	Schoettger and Mauck 1978
				LC_{50} = 2.1 (1.7 to 2.6) ppm at 12 °C, 40 ppm hardness, pH 7.5.		
				LC_{50} = 0.9 (0.7 to 1.3) ppm at 17 °C, 40 ppm hardness, pH 8.0.		
				LC_{50} = 5.4 (4.3 to 6.5) ppm at 12 °C, 320 ppm hardness, pH 8.0.		
				LC_{50} = 4.6 (3.5 to 6.0) ppm at 12 °C, 40 ppm hardness, pH 7.5.		
				LC_{50} = 3.7 (2.0 to 6.7) ppm at 12 °C, 40 ppm hardness, pH 7.5.		

Appendix C (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl (technical, 99.5%) (Cont'd)				LC_{50} = 2.1 (1.7 to 2.6) ppm at 12 °C, 40 ppm hardness, pH 8.5.		
				LC_{50} = 1.1 (0.8 to 1.6) ppm at 12 °C, 40 ppm hardness, pH 9.0.		
Carbaryl (Sevin)	Freshwater fish (Catla catla)	Surface absorption, flow-through bioassay	96 hrs	LC_{50} = 6.4 ppm (95% confidence limits = 5.0 to 8.4 ppm).	LC_{50} of metabolite, 1-naphthol, was 4.3 ppm (95% confidence limits = 4.2 to 4.4 ppm).	Tilak et al. 1981
Carbaryl (Sevin)	Freshwater fish (Mystus vittatus	Surface absorption, flow-through bioassay	96 hrs	LC_{50} = 2.4 ppm (95% confidence limits = 1.8 to 3.1 ppm).	LC_{50} of metabolite, 1-naphthol, was 1.1 ppm (95% confidence limits = 0.9 to 1.4 ppm).	Tilak et al. 1981
Carbaryl (Sevin)	Freshwater fish (Mystus cavasius)	Surface absorption, flow-through bioassay	96 hrs	LC_{50} = 4.6 ppm (95% confidence limits = 4.1 to 5.2 ppm).	LC_{50} of metabolite, 1-naphthol, was 0.33 ppm (95% confidence limits = 0.25 to 0.40 ppm.	Tilak et al. 1981
Carbaryl (Sevin)	Freshwater fish (Anabas testutus	Surface absorption, flow-through bioassay	96 hrs	LC_{50} = 5.5 ppm (95% confidence limits = 4.8 to 6.3 ppm).	LC_{50} of metabolite, 1-naphthol, was 3 ppm (95% confidence limits = 2.7 to 3.4 ppm).	Tilak et al. 1981

Appendix C (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl (Sevin)	Freshwater fish (*Labeo rohita*)	Surface absorption, flow-through bioassay	96 hrs	LC_{50} = 4.6 ppm (95% confidence limits = 3.9 to 5.3) ppm for 0.5 g fish; LC_{50} = 7.75 ppm (95% confidence limits = 6.7 to 9.0) ppm for 4.5 g fish.	For metabolite 1-naphthol, LC_{50} = 2.6 ppm (95% confidence limits = 1.8 to 3.8 ppm) for 0.5 g fish; LC_{50} = 3.13 ppm (95% confidence limits = 3.0 to 3.3 ppm) for 4.5 g fish	Tilak et al. 1979
Carbaryl (technical, 95%)	Cutthroat trout	Surface absorption, assumed to be static bioassay	96 hrs	96-hr LC_{50}'s varied with water temp., pH, and softness as follows: 6.0 (4.63 to 7.77) ppm at 7 °C, pH 7.5, soft water; 5.0 (4.10 to 6.10) ppm at 12 °C, pH 6.5, soft water; 3.95 (3.04 to 5.13) ppm at 12 °C, pH 7.5, soft water; 0.97 (0.77 to 1.22) ppm at 12 °C, pH 8.5, soft water; 3.95 (3.37 to 4.63) ppm at 12 °C, pH 7.8, hard water.	95% confidence limits in parentheses. Fish are more sensitive as temp. is increased and as pH is increased. Toxicity given in terms of parts active ingredient.	Woodward and Mauck 1980
Carbaryl (49% formulation)	Cutthroat trout	Surface absorption, assumed to be static bioassay	96 hrs	LC_{50} = 6.7 (5.23 to 8.60) ppm.	Toxicity given in terms of parts active ingredient; 95% confidence limits in parentheses. Temp. 12 °C, pH 7.5, soft water.	Woodward and Mauck 1980

Appendix C (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl (50% WP)	Air breathing fish (*Macropodus cupanus*)	Surface absorption, static bioassay	26 days	96-hr LC_{50} = 3.5 ppm, (100% survival at 96 hrs with 2.5 ppm, 100% mortality with 4.5 ppm).	Sublethal conc. (1.0, 1.5, 2.0, 2.5 ppm) resulted increased rate of surfacing and swimming; no change in feeding rate or food absorption rate; decreased growth rate; increased metabolic rate; and decreased food conversion efficiency, compared to controls.	Arunachalam and Palanichamy 1981
Carbaryl	Freshwater fish (*Tilapia* sp.)	Surface absorption, static bioassay	48 hrs	LC_{50} = 5.495 (4.40 to 6.59) ppm.	95% confidence limits in parentheses. Water temp. of 26 to 28 °C, pH 7.0 0.2, and hardness 140 to 220 ppm.	Basha et al. 1983
Sevin (99% a.i.)	Fish (*Puntius ticto*)	Surface absorption, static bioassay	96 hrs	24-, 48-, 72-, and 96-hr TL_{50}'s were 4.2, 3.9, 3.8, and 3.7 ppm.	Temp. varied from 10.5 to 29 °C; pH 7.6 to 8.3, hardness 68 to 88 ppm.	Bhatia 1971
Carbaryl (Sevin; 100% purity)	Channel catfish	Surface absorption, static bioassay	24, 48, and 96 hrs	In filtered tap water, the 24, 48, 96-hr median.	Values in parentheses are 95% confidence limits.	Brown et al. 1979

Appendix C (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl (Sevin; 100% purity) (Cont'd)				toxic limits (TLM) were 6.71 (5.89 to 7.78) ppm; 1.30 (1.24 to 1.40) ppm and 1.30 (1.24 to 1.70) ppm.	Filtered water at pH 8.5, 597 ppm total salts, and 23 °C (± 2 °C). In two samples of rice paddy water, the 24-hr TLM was 2.27 and 6.02 ppm; the 48-hr TLM was 1.53 and 2.00 ppm; and the 96-hr TLM was 0.14 and 1.56 ppm.	
Carbaryl (94% a.i.)	Common carp (*Cyprinus carpio*)	Surface absorption, static bioassay	96 hrs	24-hr LC_{50} = 10.0 ppm; 96-hr LC_{50} = 1.7 (0.99 to 2.93) ppm.	95% confidence limits in parentheses. 8-wk old fish (0.38 g) were used. Temp. 29 ± 3 °C; pH 7.0 0.1; hardness = 22 ppm as $CaCO_3$.	Chin and Sudderuddin 1979
Sevin (50% WDP)	Tilapid fish (*Sarotherodon mosambica*)	Surface absorption, static bioassay	72 hrs	LC_{50}'s were 13, 10, and 8 ppm for 24, 48, and 72-hr exposures.	Fish tested at 28 to 30 °C, water pH = 7.0.	Koundinyo and Ramamurthi 1980
Carbaryl (99%)	Freshwater fish (*Tilapia* sp.)	Surface absorption	48 hrs	TL_{50} = 1.9585 (1.8945 to 2.0621) ppm.	95% confidence limits in parentheses.	Li and Chen 1981
Carbaryl (99%)	Mosquito fish (*Gambusia patruelis*)	Surface absorption	48 hrs	TL_{50} = 0.736 (0.540 to 0.955) ppm.	95% confidence limits in parentheses.	Li and Chen 1981

Appendix C (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl	Estuarine teleost (*Therapon jarbua*)	Surface absorption, static bioassay	96 hrs	LC_{50} = 2.20 (1.66 to 2.90) ppm.	95% confidence limits in parentheses. Water temp. 29 ± 1 °C; pH 7.9; salinity 19.21 pp thousand. Fish exposed to LC_{25} (0.62 ppm) had elevated respiratory rate compared to controls for 8 hrs of observation.	Lingaraja and Venugopalan 1978
Carbaryl (Sevin; 50% WP)	Freshwater Cyprinnid fish (*Puntius conchonius*)	Surface absorption, bioassay	Up to 30 days.	96 hr LC_{50} = 2.142 2.142 ppm commercial formulation. Exposed fish had significant hyperglycemia and glycogenolysis in liver, brain, and heart.	Long term exposure (15 to 30 days) to 0.194 and 0.306 ppm resulted in hypoglycemia and depletion of liver glycogen.	Pant and Singh 1983
Carbaryl	Rainbow trout	Surface absorption, closed circulation system.	Not specified; at least 5 1/2 hrs.	Fish died within 4 1/4 to 5 1/2 hrs when exposed to 1.5 ppm.	Fish weighed 150 to 250 g.	Van Hoof 1980
Carbaryl	Carp (*Cyprinus carpio*)	Surface absorption, static bioassay	96 hrs	TL_{50} = 2.0 ppm. The estimated maximum acceptable toxicant conc. was 0.050 to 0.080 ppm.	—	Verma et al. 1981

Appendix C (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl (Sevin; 50% WP)	Carp (*Cirrhina mrigala*	Surface absorption, static bioassay	96 hrs 60 days	96 hr LC_{50} = 1.94 ppm. Maximum allowable toxicant conc. (MATC) was 0.0873 to 0.1086 ppm based on 60 days of exposure to 0.050; 0.074; 0.098; 0.122; 0.146; 0.170; 0.194; 0.218; or 0.242 ppm, representing 1/8 to 1/40 of the 96-hr LC_{50}.	MATC was equivalent to a no observed effect conc.	Verma et al. 1984
Carbaryl (50% WP)	Catfish (*Mystus vittatus*)	Surface absorption, static bioassay	72 hrs	LC_{50} = 17.5 ppm. At 32.5 ppm, 100% mortality occurred within 24 hrs, at 12.5 ppm or less, there were no mortalities within 72 hrs.	Fish weighed 1.10 g. Tested at 28 °C. Sublethal concs. resulted in accelerated swimming activity and increased frequency of opercular beating. A 27-day exposure to sublethal concs. resulted in decrease feeding and slower growth.	Arunachalam et al. 1980
Carbaryl (80% WP)	Mosquito fish (*Gambusio affinis*)	Surface absorption, static bioassay	96 hrs	TL_{50}'s at 24, 48, 72, and 96 hrs were 40.00 (42.50), 35.00 (36.20), 32.40 (33.60), and 31.80 (32.80) ppm.	Values in parentheses are for carbaryl in acetone.	Chaiyarach et al. 1975

Appendix C (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl (97%)	Fish (Lebistes reticulatus)	Surface absorption, static bioassay	96 hrs	12-, 24-,48-, 72-, and 96-hr LC_{50}'s were 39.20, 17.70, 11.20, 10.30, and 9.74 ppm. The 12-, 24-, 48-, 72-, and 96-hr PC_{50}'s (paralysis or death of 50%) were 16.90, 12.50, 10.10, 9.74, and 9.74 ppm.	Threshold conc. for distinct pathology was 6.2 ppm.	Lejczak 1977
Carbaryl (50% WP)	Freshwater teleost (Channa punctatus	Surface absorption, static bioassay	96 hrs	24,- 48-, 72-, and 96-hr LC_{50}'s were 21.20, 20.50, 20.02, and 19.50 ppm.	Fish weighed 24.5± 2.6 g. Displayed restlessness erratic opercular movements, convulsions, and died on bottom.	Singh et al. 1984
Carbaryl (50% WP)	Freshwater teleost (Meteropneustes fossilis)	Surface absorption, static bioassay	96 hrs	24-, 48-, 72-, and 96-hr LC_{50}'s were 22.95, 22.30, 21.45, and 20.10 ppm.	Fish weighed 23.0 3.5 g. Displayed restlessness, erratic opercular movements, convulsions, and died on bottom.	Singh et al. 1984
Carbaryl (50% WP)	Carp (Cyprinus carpio communis)	Surface absorption, static bioassay	72 hrs	24-hr LC_{50} = 13.51 ppm; 48-hr. LC_{50} = 11.74 ppm; and 72-hr LC_{50} = 10.36 ppm.	At 13.50 ppm, test fish showed excitation, surfacing, vertical guivering, incrased opercular beating, slow swimming on their sides, decreasing rate of respiration drowning at the bottom of the tank.	Toor and Kaur 1974

Appendix C (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl (Sevin; 50% WP)	Singii (*Saccobranchus fossilis*; Cypriniformes)	Surface absorption, static bioassay	Up to 96 hrs	24-hr LC_{50} = 23.01 ppm; 48-hr LC_{50} = 20.80 ppm; 72-hr LC_{50} = 19.79 ppm; 96-hr LC_{50} = 19.58 ppm.	Water temp. was 18 ± 2 °C; pH was 7.2 ± 0.2. Fish were 50 to 75 mm long and 5 to 10 g in weight.	Verma et al. 1982
Carbaryl	Pinfish	Surface absorption, flow-through system	15 hrs	At 1.3 ppm, mean activity of AChE was reduced 79% compared to controls, from 2 μmol to acetycholine hydrolyzed/hr/mg of brain tissue.	Reduction of activity was significant at $p < 0.005$. Carbaryl conc. used resulted in death of 40 to 60% of the test fish. No dead fish were used for bioassay.	Coppage 1977
Carbaryl	Spot	Surface absorption, flow-through system	Up to 5.0 mos.	Fish exposed to 0.1 ppm showed 17% inhibition of brain AChE activity after 2.5 mos and after 5.0 mos. Exposure to 1.0 ppm for 13 days resulted in 32% inhibition of activity.	Started with juvenile (18 mm) fish. Average monthly water temp. varied from 16 to 29 °C, salinities from 24 to 30 pp thousand.	Lowe 1967

Appendix C (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Sevin	Tilapid fish (*Sarotheroden mossambica*)	Surface absorption	Not given.	Exposure to 10 ppm (lethal conc. resulted in a significant ($p < 0.001$) decrease in AChE activity increase in acetylcholine content in brain, gill, muscle, liver intestine, and kidney.	—	Koundinya and Rama-Murthi 1979b
Carbaryl (Sevin-4-oil)	Brook trout (*Salvelinus fontinalis*)	Surface absorption, field situation	Up to 4 wks after 2-sec. application.	Application of 0.5 lb/acre twice at 7-day interval. Mean brain AChE activity was significantly ($p = 0.05$) lower in fish from treated watershed 3 days after first application and day of sec. application, but depression disappeared within 24 to 48 hrs. *In situ* test (fish caged in stream) indicated no treatment-realted mortalities.	—	Haines 1981

Appendix C (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl	Fathead minnow	Surface absorption, flow-through bioassay	9 mos (through one life cycle)	Fish exposed to 0, 0.008, 0.017, 0.062, 0.21, or 0.68 ppm. 96-hr TL_{50} and LTC (lethal threshold concentration) were 9.0 ppm. MATC (minimum acceptable toxicant toxicant conc. at pH 7.5 and 45.2 ppm hardness between 0.21 and 0.68 ppm carbaryl.	At 0.68 ppm, survival was decreased and reproduction inhibited (retained sperm and apperent resorptive ovaries). TL_{50} and LTC for 2-mo-old minnows.	Carlson 1971
Carbaryl	Carp (Cyprinus communis)	Surface absorption, static bioassay	Through hatching.	LC_{50} for hatching was 1.4 ppm, for fry was 10.36 (specific time not given). Carbaryl at 2.5 ppm reduced hatching to 0%, at 1.0 ppm, 76.7% of eggs hatched (95% of controls hatched).	Deformities included enlargment of pericardial sac, circulatory failure and curling of the posterior.	Kaur and Toor 1977
Sevin (50% WP)	Freshwater teleost (Channa striatus)	Surface absorption, static conditions	30 days	Sublethal conc. (10 and 20 ppm) severely affected reproductive systems. Effects included decreased percentage of mataring and mature oocytes, rupture of oocyte walls, and dilation of blood vessels.	—	Kulshrestha and Arora 1984

Appendix C (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl	Medaka (Oryzias latipes)	Surface absorption, static bioassay	Fertilization through hatching.	Circulatory system anomalies such as rudimentary heart development, pericardial edema, irregular heartbeat, backflow of blood and clots in atria, and extra embryonic circulation observed in more than 80% of eggs exposed to 5 ppm carbaryl.	ED_{50} was 2.5 ppm.	Solomon 1977 and Solomon and Weis 1979
Carbaryl (technical, 99%)	Killifish (Fundulus heteroclitus)	Surface absorption, static bioassay	Various.	Early embryos exposed to 10 ppm carbaryl showed developmental arrest prior to initiation of heartbeat.	Most embryos recovered if removed after 3 days of exposure. Longer exposure resulted in heart malformations and no formation of blood pigment.	Weis and Weis 1974a
Sevin	Tilapid fish (Sarotherodon mossambica)	Surface absorption	Not given.	Exposure to 10 ppm (lethal conc. resulted in a significant decrease in red blood cell count ($p < 0.05$); packed cell volume ($p < 0.001$); and hemoglobin conc. ($p < 0.001$). ($p < 0.001$).	Nonsignificant increases in mean corpuscular volume, and mean corpuscular hemoglobin conc. were also reported.	Koundinya and Ramamurthi 1979a

Appendix C (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Sevin	Tilapid fish (*Sarotherodon mossambica*)	Surface absorption	30 days	Exposure to 4 ppm (sublethal conc.) resulted in a significant ($p < 0.001$) increase in red blood cell count, packed cell volume, and hemoglobin conc.	Nonsignificant decreases in mean corpuscular hemoglobin and its conc. and increase in mean corpuscular volume were also reported.	Koundinya and Ramamurthi 1980a
Carbaryl	Freshwater teleost (*Channa punctatus*)	Surface absorption, static bioassay with 24-hour renewal.	60 days	Fish exposed to 1.05 ppm had decreased liver and muscle glycogen; increased liver and muscle lactic acid; higher activity of lactate dehydrogenase in liver, muscles, brain, and gills; decreased LDH in kidney and intestine; decreased pyruvate dehydrogenase activity in all tissues measured; decreased succinate dehydrogenase activity in muscle, liver, and brain; and increased succinate dehydrogenase activity in kidney and intestine.	Pattern of effects indicates anaerobic metabolism favored and aerobic metabolism favored and aerobic metabolism impaired.	Sastry and Siddiqui 1982

Appendix C (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl (technical, 99%)	Killifish (*Fundulus heteroclitus*)	Surface absorption, static bioassay	2 wks	0.01 ppm resulted in significantly ($p = 0.005$) reduced regeneration in caudal fin after 1 wk in 1 of 2 replicates and significantly ($p = 0.01$ and $p = 0.001$) reduced regeneration after 2 wks in 2 of 2 replicates.	—	Weis and Weis 1975
Carbaryl (Sevin; 98% a.i.)	Sheepshead minnows	Surface absorption, double Y-maze flow-through chamber.	1 hr	Fish did not avoid ($p < 0.05$) carbaryl at concs. of 10.0, 1.0, or 0.1 ppm (24-hr LC_{50} = 2.8 ppm.	Fish 20 to 40 mm in length were tested in diluted filtered sea water at 20 pp thousand salinity and 20 °C.	Hansen 1969
Carbaryl (Sevin; 98% a.i.)	Mosquitofish	Surface absorption, double Y-maze flow-through chamber.	1 hr	Fish selected fresh water (60.4% of total) in preference to water with 10 ppm carbaryl ($p < 0.05$)	Fish 20 to 45 mm long were tested at 20 °C. Exposure for 24 hrs to 10 ppm caused "irritation."	Hansen et al. 1972
Carbaryl (Sevin; 95% purity)	Atlantic salmon (juvenile)	Surface absorption, static conditions.	24 hrs	Exposure to 0.25 to 1.00 ppm did not change the temp. preference of the test fish in a horizontal temp. gradient.	Test fish were 3.0 to 4.0 cm in length and acclimated for at least 3 wks at 15 °C.	Peterson 1976

Appendix C (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl (technical, 99.2%)	Fish (Menidia menidia)	Surface absorption, static bioassay	72 hrs	Single application of 0.100 ppm disrupted schooling behavior within 24 hrs of exposure. Fish placed into clean water did not return to normal behavior until 72 hrs.	Disruption of schooling and its continuance in clean water may be due to accumulation of the toxic metabolite, 1-naphthol.	Weis and Weis 1974b
Carbaryl (50%)	Bullfrog (Rana catesbicana; Amphibia)	Acute oral by capsule.	Observed for 14 days after single dose.	LD_{50} >4,000 mg/kg	—	Tucker and Crabtree 1970
Carbaryl	African clawed frog (Xenopus laevis; Amphibia)	Surface absorption in embryos.	24 hrs	LC_{50} = 4.7 ppm (3.9 to 5.6 ppm). EC_{50} (abnormalities leading to post hatching death) = 0.11 ppm (0.08 to 0.14 ppm).	95% confidence limits in parentheses. Tested at 18 °C. Embryos treated with 10 ppm prior to gastrulation showed internal abnormalities, such as reduced or absent heart, occlusions of notochoral or spinal cord, yolk platelet lyses, and edema.	Elliott-Feeley and Armstrong 1982

Appendix C (Concluded)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl (Karbatox 75)	Frog (*Rana temporaria*; Amphibia)	Surface absorption, with renewal every other day.	Up to 10 days.	Exposure to 0.1% resulted in 100% mortality within 10 to 12 hrs, exposure to 0.05% resulted in 100% mortality within 20 to 24 hrs, exposure to 0.001% resulted in 90% mortality within 7 to 10 days and of the survivors within 2 days of transfer to fresh water.	Exposed animals exhibited hyperactivity followed slow swimming and sinking to the tank bottom. Abnormalities included muscular abnormality at the base of the tail.	Rzehak et al. 1977
Carbaryl (Karbatox 75)	African clawed frog (*Xenopus laevis*; Amphibia)	Surface absorption, with renewal every other day.	Up to 8 days.	Exposure to 0.01% for 24 hrs resulted in 80% mortality within 1 wk after return to fresh water; remaining died within 3 wks. Exposure to 0.001% for 8 days resulted in 20% mortality 1 wk after return to fresh water and 65% mortality 2 wks after return.	Exposed animals exhibited hyperactivity followed by slow swimming and sinking to the tank bottom. Abnormalities included muscular abnormality at the base of the tail.	Rzehak et al. 1977

Appendix D

Summary of Carbaryl Toxicity Data for Birds

Appendix D

Summary of Carbaryl Toxicity Data for Birds

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl (50% WP)	Chickens (Alexandria-cross hens)	Acute oral dose by capsule.	Observed for 24 hrs after single dose.	450 mg/kg resulted in 100% mortality. LD_{50} = 248 mg/kg. Chickens were dosed with 0, 100 150, 250, 350, and 450 mg/kg body weight.	Chickens were 3 1/2- to 4 1/2-mo-old females. After dosing, birds became quiescent, were unable to stand, had diarrhea, and displayed tremors just before death.	El-Dakoury et al. 1979
Sevin	Redwing blackbird	Acute, by gavage.	Not given.	LD_{50} = 56 mg/kg	95% confidence limits = 32 to 100 mg/kg.	Schafer 1972
Carbaryl	Bobwhite	Single dose in corn-oil suspension into crop.	Observed 2 days and 8 days after treatment.	Birds fed 10, 50, or 90 mg/kg in 1.0 ml corn-oil did not exhibit significant changes in body weight, energy intake, energy excretion, energy metabolized, or metabolic efficiency compared to controls.	—	Solomon and Robel 1980
Carbaryl (100%)	Mallards (females)	Acute oral dose by capsule.	Observed for 14 days after single dose.	$LD_{50} > 2,179$ mg/kg.	Values for 3 mo-old animals. Some reguritation observed. A 30-day "empirical minium lethal dose" was about 125 mg/kg/day.	Tucker and Crabtree 1970

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl (95%)	Pheasants (males)	Acute oral dose by capsule.	Observed for 14 days after single dose.	$LD_{50} > 2,000$ mg/kg	Values for 3- to 4-mo-old animals.	Tucker and Crabtree 1970
Carbaryl (4 lb/gal.)	Pheasants (females)	Acute oral dose by capsule.	Observed for 14 days after single dose.	$LD_{50} = 707$ mg/kg	Values for 3- to 4-mo-old animals.	Tucker and Crabtree 1970
Carbaryl (85%)	Coturnix quail (males)	Acute oral dose by capsule.	Observed for 14 days after single dose.	$LD_{50} = 2,290$ mg/kg	95% confidence limits 1,740 to 3,020 mg/kg. Values for 2 mo-old animals.	Tucker and Crabtree 1970
Carbaryl (85%)	Pigeon (males and females)	Acute oral dose by capsule.	Observed for 14 days after single dose.	$LD_{50} > 1,000$ to 3,000 mg/kg	—	Tucker and Crabtree 1970
Carbaryl (95%)	Sharp-tailed grouse (females)	Acute oral dose by capsule.	Observed for 14 days after single dose.	$LD_{50} > 780$ to 1,700 mg/kg	Values for 3- to 12 mo-old animals.	Tucker and Crabtree 1970
Carbaryl (100%)	Canada geese (males and females)	Acute oral dose by capsule.	Observed for 14 days after single dose.	$LD_{50} = 1,790$ mg/kg	95% confidence limits 1,480 to 2,180 mg/kg.	Tucker and Crabtree 1970
Carbaryl	Coturnix quail	Single oral dose by capsule.	Eggs collected 6 days before and 6 days after dosing.	Dose of 1,000 mg/kg caused no appreciable egg-shell thinning (<5 μm reduction between pre- and post-treatment thickness).	Six animals tested.	Haegle and Turner 1974

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl	Mallards	Single oral dose by capsule.	Eggs collected 6 days before and 6 days after dosing.	Dose of 1,000 mg/kg caused no appreciable egg-shell thinning (<5 μm reduction between pre- and post-treatment thickness).	Five animals tested.	Haegle and Turner 1974
Carbaryl (5%)	Japanese quail	Dermal, dusting.	Animals observed for 24 hrs.	No overt signs of toxicity were reported. Some reduction in plasma ChE activity was observed by 1.5 hrs, this reduction was significant ($p < 0.05$) by 6 hrs, and returned to normal for females by 24 hrs. Brain ChE was not altered by dusting with 5% material.	Exposures calculated to be 160 mg/kg for males and 140 mg/kg for females.	Hill 1979
Carbaryl	Chicken	Acute subcutaneous injection. Chickens first given 15 mg atropine/kg body weight for protection against acute poisoning.	Up to 24 days observation.	Lowest lethal dose tested was 800 mg/kg. Neurotoxic effects (leg weakness, paralysis) were observed, with the highest ineffective dose test tested at 800 mg/kg and the lowest effective dose tested at 1,600 mg/kg.	Neurotoxic effects observed within 24 hrs of dosing, for up to 24 days.	Gaines 1968

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl (technical, 99%)	Japanese quail	Subchronic, oral.	From hatching through 14 wks of age.	Animals were fed 0, 50, 150, 300, 600, 900, and 1,200 ppm in feed. Males fed 900 and 1,200 ppm had decreased body weights, increased relative brain weights, and increased relative liver weights, compared to controls. Animals fed 150, 600, 900, and 1,200 ppm had increased kidney weights. Only females fed 900 ppm had significantly decreased body weights from controls. Females fed 900 and 1,200 ppm had higher kidney weights.	No significant effects on egg production or hatchability were observed, although there was a slight decrease in egg production at 600, 900, and 1,200 ppm levels. Gross teratogenic effects were not observed.	Bursian and Edens 1977
Carbaryl (technical, 99%)	Japanese quail	Oral in feed.	From hatching through 14 wks.	Dose levels of 0, 50, 150, 300, 600, 900, and 1,200 ppm had no effect on whole-brain AChE activity, whole-brain and heart norepine-phrine and epine-phrine levels, and plasma glucose levels. Whole	—	Bursian and Edens 1979

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl (technical, 99%) (Cont'd)				brain dopamine levels were significantly elevated in males fed 600 and 1,200 ppm. No effects were noted among females.		Burisan and Edens 1979
Carbaryl	Bobwhite	Oral	Fed carbaryl in diet for 5 days and observed for 3 days more on untreated diet.	$LC_{50} > 2{,}400$ ppm	No mortality to 2,400 ppm. Birds were 14 days old at start of feeding. A total of 6 (unspecified) concs. were used.	Hill et al. 1975
Carbaryl	Japanese quail	Oral	Fed carbaryl in diet for 5 days and observed for 3 days more on untreated diet.	$LC_{50} > 5{,}000$ ppm	No mortality to 2,400 ppm. Birds were 7 days old at start of feeding. A total of 3 (unspecified) concs. were used.	Hill et al. 1975
Carbaryl	Ring-necked	Oral	Fed carbaryl in diet for 5 days and observed for 3 days more on untreated diet.	$LC_{50} > 5{,}000$ ppm	No mortality to 5,000 ppm. Birds were 23 days old at start of feeding. A total of 3 (unspecified) concs. were used.	Hill et al. 1975

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl	Mallard	Oral	Fed carbaryl in diet for 5 days and observed for 3 days more on untreated diet.	LC_{50} >5,000 ppm	No mortality to 5,000 ppm. Birds were 24 days old at start of feeding. A total of 3 (unspecified) concs. were used.	Hill et al. 1975
Opigal (Kaolin powder with 50% Sevin)	Hens (white Leghorn)	Oral by capsule daily.	60 days	Hens were given equivalent of 180 or 540 mg Sevin/kg body weight. No mortalities observed after 60 days of exposure to 180 mg/kg; 100% mortality occurred within 35 days of exposure to 540 mg/kg.	In low-dose group, no changes were noted in appearance, behavior, quantity or quality of eggs laid, hematology, or histopathology. Some decrease in food consumption was observed during the first 2 wks. No changes in blood chemistry or hemotocrit. In high dose group, hens were restless after 3 days, had intense diarrhea, sharply decreased food consumption, fatty degeneration of liver, interstitial myocarditis, degenerated ovaries, fibrous atrophy of spleen, and enlargement of gall bladder. No changes in blood chemistry or hematocrit through day 21.	Nir et al. 1966

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Ortho liquid (Sevin, carbaryl)	Mallard (eggs)	Surface absorption (egg immersion)	Immersed for 30 secs. on day 3 of incubation. Living embryos removed from egg on day 18 of incubation.	In aqueous medium LC_{50} >equivalent of 216 lb/acre at 100 gal/acre >emulsifiable conc.) and >36 x field application levels. In oil carrier, LC_{50} 24 lb/acre at 11 gal/acre and >20 x field application levels at 100 gal/acre. In oil, LD_{50} 118 µg/g egg.	No malformations with aqueous carrier. In oil carrier, of growth reduced and no. of abnormal survivor survivors significantly different from controls.	Hoffman and Albers 1984
Carbaryl	Chicken (embryo)	Not given	Not given	1 mg/egg produced malformations.	--	Marliac 1964
Carbaryl	Chicken (fertile white leghorn eggs)	Injected into the yolk on day 4 or 5 of incubation.	Some eggs tested biochemically at day 6 to 12 of incubation. At least 10 eggs incubated for 19 days then examined for teratogenic signs.	Eggs injected with doses of 0.1, 0.3, 1.0, or 3.0 mg/egg. Body weight 0.93, 0.91, 0.86, and 0.75 that of controls; body length 0.94, 0.95, 0.96, and 0.90 of controls; and leg length 0.92, 0.87, 0.72, and 0.61 of controls for eggs dosed with 0.1, 0.3, 1.0, and 3.0 mg.	Eggs weighed 45 to 55 g and were incubated at 37 °C. Levels of nicotinamide adenine dinucleotide in treated eggs were less than controls, with a dose-related depression indicating an association between depressed levels of NAD and teratogenesis.	Proctor and Casida 1975; Proctor et al. 1976

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl				Incidence of straight legs was 0, 11, 28, and 50%; of abnormal feathers 11, 22, 72, and 100%; and of wry neck 0, 0, 0, and 0% at doses of 0.1, 0.3, 1.0, and 3.0 mg/egg.		Proctor and Casida 1975; Proctor et al. 1976
Carbaryl	Chicken (embryos)	Injected into egg.	5 and 12 days	Eggs injected with 1.0, 2.5, and 5.0 mg in either acetone or sesame oil vehicle and 10.0 mg in acetone before incubation. Embryos examined at 5 or 12 days of incubation and survivors examined for abnormalities. Significant increases in mortality after exposure to carbaryl for 5 and 12 days compared to exposure to vehicle alone.	No external abnormalities were observed among embryos exposed for 5 days. Embryos exposed for 12 days showed accumulations of fluid below the epidermis (1 of 13 survivors at 10.0 mg in acetone; 1 of 10 survivors at 1.0 mg in sesame oil). Other malformations included cleft foot, tonically flexed digits, and unilateral ophthalmia.	Swartz 1981

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl	Bobwhite	Oral, in diet.	Fed 7 days in diet and observed for 14 days after treatment.	There was no statistically significant difference in weight of food consumed; weight of birds; or daily locomotor activity of birds during pretreatment (35 days); treatment (7 days); or post-treatment (14 days) among birds treated with 237 or 1,235 ppm in diet.	Concentration chosen represented estimated ranges of exposure for adult bobwhite from foliar application during late spring, early summer agricultural operations in Kansas. Eight birds tested at each dose level.	Robel et al. 1982
Carbaryl	Bobwhite (adult males)	Oral	15 days	Ingestion of 4, 18, or 30 mg/kg/day of carbaryl in feed did not alter fat reserves or body weights of underweight animals compared to controls.	—	Rodgers and Robel 1977
Sevin	Bobwhite	Oral, by pipette.	Up to 25 days	Low dosages increased susceptibility to infection by parasitic protozoan Histomonas meleagridis. Quail tolerated 50 μg of carbaryl but as much as 10 μg led to death from histomiasis.	—	Zeakes et al. 1981

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl	Chick	Injection	—	LD_{50} = 3.44 mg/embryo following injection into allantoic cavities of 10 to 11-day-old embryos.	No histopathological changes in organs were observed.	Tos-Luth et al. 1973
Sevin (85% WP)	Japanese quail	Injection into air space on day 9 of incubation.	Observed until day 14 of incubation.	Eggs treated with 0.01, 0.10, or 1.0 mg/egg. Mean embryonic weights on day 14 were 4.18, 5.70, 4.68, and 3.67 g for controls and for eggs treated with 0.01, 0.10, or 1.0 mg/egg. One each of the 0.10 and 1.0 mg/egg group died during days 0 to 8 and days 9 to 10. No deaths were observed during days 11 to 14.	One egg of 10 treated with 0.10 g exhibited defective development of skull and cervical vertebrae.	Varnagy 1981
Sevin	Natural bird populations in in New Jersey	Field application	Approximately 6 wks.	Application of 1 lb/acre twice resulted in a significant decrease in numbers of individual birds numbers of species and species diversity compared to controls.	—	Moulding 1976

Appendix D (Concluded)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl	Domestic turkeys	Application in turkey houses to control lesser mealworm.	NA	Application of carbaryl at 14 x recommended dose in turkey house resulted in 16.4% mortality at 10 days. Levels of 1.7 and 3.6 ppm carbaryl were detected in skin and fat.	10 lbs. carbaryl in 25 gal water applied to 1,000 sq. ft. of turkey house twice at 4-day intervals. Second application also sprayed on the birds and contaminated both water. No histological effects in autopsied birds.	Bahl and Pomeroy 1978

NA = data not available.

Appendix E

Summary of Carbaryl Toxicity Data for Mammals

Appendix E

Summary of Carbaryl Toxicity Data for Mammals

Formulation	Organism	Nature of Exposure	Effects	Comments	Reference
			Acute Oral Toxicity		
Carbaryl (purity unspecified)	Rat (female)	Oral dosing	LD_{50} = 500 mg/kg	--	Gaines 1969, in Kuhr and Dorough 1976
Carbaryl (purity unspecified)	Rat (male)	Oral dosing	LD_{50} = 850 mg/kg	--	Gaines 1969, in Kuhr and Dorough 1976
Carbaryl (purity unspecified)	Mouse	Oral dosing	LD_{50} = 438 mg/kg	--	Rybakova 1966, in International Agency for Research on Cancer 1976
Carbaryl (purity unspecified)	Rabbit	Oral dosing	LD_{50} = 710 mg/kg	--	Carpenter et al. 1961
Carbaryl (purity unspecified)	Man	Ingestion of 250 mg (about 2.8 mg/kg carbaryl.	Twenty mins after ingestion, man experienced sudden onset of violent epigastric pain and a little later he began to sweat profusely. He gradually developed great lassitude and vomited twice. One hour after ingestion and following a total dose of 3 mg of atropine, he was feeling better. After a sec. hr, he was completely recovered.	--	Hayes 1982

Formulation	Organism	Nature of Exposure	Effects	Comments	Reference
Acute Intraperitoneal Toxicity					
Carbaryl (purity unspecified)	Rat	Intraperitoneal administration	LD_{50} = 200 mg/kg	--	Hayes 1982
Carbaryl (purity unspecified)	Mouse	Intraperitoneal administration	LD_{50} = 25 to 29 mg/kg	--	Hayes 1982
Carbaryl (purity unspecified)	Rabbit	Intraperitoneal administration	LD_{50} = 223 mg/kg	--	Hayes 1982
Acute Dermal Toxicity					
Carbaryl (purity unspecified)	Rat (male or female)	Dermal dosing	LD_{50} = greater than 4 g/kg	--	Gaines 1969, in Kuhr and Dorough 1976
Acute Intravenous Toxicity					
Carbaryl (purity unspecified)	Rat	Intravenous administration	LD_{50} 24 to 42 mg/kg	--	Hayes 1982
Eye Irritation					
Carbaryl (technical)	Rabbit	Apply 0.5 ml of a 10% suspension of carbaryl in proplylene glycol to the cornea of 1 eye for each of 5 rabbits.	Mild injury on 1 of 5 eyes.	--	Carpenter et al. 1961

Appendix E (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	Reference
			Eye Irritation (Concluded)		
Carbaryl (technical)	Rabbit	A 25% aqueous suspension of of carbaryl.	Caused no injury.	--	Carpenter et al. 1961
Carbaryl (technical)	Rabbit	50 mg of dust.	Caused traces of corneal necrosis.	--	Carpenter et al. 1961
			Inhalation Toxicity		
Carbaryl (50% WP)	Guinea pig	Group of 6 guinea pigs inhaled carbaryl for 4 hrs at 390 mg/m^3.	Nasal and ocular irritation. Healed hemorrhagic areas in lungs found at autopsy at 14 days.	Average particle size of 15 μm.	Carpenter et al. 1961
Carbaryl (85% WP)	Guinea pig	Group of 6 guinea pigs inhaled carbaryl for 4 hrs at 230 mg/m^3.	During 14-day observation, animals showed slight weight decrease but regained pretreatment weight by the end.	Average particle of 5 μm.	Carpenter et al. 1961
Carbaryl (95% water WP)	Man	Carbaryl applied by 10 spraymen as 5% spray inside 16 houses at rate of 2 g/m^2. Application time was 6 hrs.	Only clinical effect observed was skin rash in sprayman whose back was splashed accidentally with carbaryl. Spraymen showed no increase in excretion of 1-naphthol (metabolite of carbaryl) but 63 villagers sampled 1 wk after spraying showed significant increase in excretion of 1-naphthol.	--	Vandekar 1965, in Hayes 1982

Appendix E (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	Reference
Subchronic Toxicity					
Carbaryl (purity unspecified)	Rat (10 males and 10 females)	Fed carbaryl a 0, 1,500 or 2,500 ppm in the diet for 96 days.	Kidney weight of females at both 1,500 or 2,500 ppm increased. There was a minor degree of diffuse, cloudy swelling of kidney tubules 4 of 10 females fed 2,500 ppm of carbaryl.	Authors speculated that apparent sex difference in response occurred because dose for females was about 30% greater than that for males at the same dietary intake.	Carpenter et al. 1961
Carbaryl (purity unspecified)	Man	Ingestion of carbaryl daily for 6 wks at 0.06 mg/kg by 5 men or 0.12 mg/kg by 6 men.	Following tests were normal at both dosages: BSP, EEG, plasma and erythrocyte ChE, complete blood count, and blood chemistry. Urine showed abnormality attributable to carbaryl at higher dosage; also ratio of amino acids to cretinine nitrogen in the urine decreased.	Interpreted as indication of slight, reversible decrease in ability of proximal convoluted tubules to reabsorb amino acids.	Wills et al. 1968
Carbaryl (85% WP)	Rat	A group of rats (number unspecified inhaled carbaryl at a conc. of 10 mg/m^3, 7 hrs/day, 5 days/wk, for a total of 90 inhalation periods.	No mortality nor grossly visible injury were observed among the exposed animals.	--	Carpenter et al. 1961

Appendix E (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	Reference
			Chronic Toxicity		
Carbaryl (purity unspecified)	Rat (20 males and 20 females/dose, 2-mos old)	Dietary feeding of carbaryl at 50, 100, 200 or 400 ppm for 2 yrs.	NOEL = 200 ppm. Cloudy swelling of hepatic cords located around the central veins animals on 400-ppm diet for 2 yrs.	--	Carpenter et al. 1961
Carbaryl (purity unspecified)	Rat (24 males and 24 females/dose)	Rats were administered carbaryl orally at 7, 14, or 70 mg/kg for up to 1 yr. (Method of administration not clear.)	Functional and structural changes of pituitary gland and impairment of thyroid and gonadal function in rats all all dose levels.	--	Shtenberg and Rybakova 1968, in U.S. Department of Health, Education, and Welfare 1976
			Reproduction and Teratogenic Effects		
Carbaryl (purity unspecified)	Rat	Carbaryl administered by peroral intubation daily for 12 mos at 2, 5, or 15 mg/kg/day prior to mating rats.	NOEL for reproductive effects in rat was 2 mg/kg. Reproduction at 5 or 15 mg/kg (details not given) were reduced (details unspecified).	--	Orlova and Zhalbe 1968, in Weil et al. 1972
Carbaryl (99.8% pure)	Rat (Carworth Farms Elias)	Carbaryl fed to rats at dietary levels of 0, 2.5, or 10 mg/kg for generations.	No statistically significant dose-related effect on fertility, gestation, viability of pups, or lactation.	--	Weil et al. 1972

Appendix E (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	Reference
			Reproduction and Teratogenic Effects (Continued)		
Carbaryl (purity unspecified)	Rats (Osborne-Mendel)	Carbaryl was fed to rats at dietary levels of 2,000, 5,000, or 10,000 ppm (equivalent to to 100, 250, or 500 mg/kg/day for three generation.	Reproductive effects at all doses. Decreases were seen in fertility, viability, and lactation indices in all litters at 10,000 ppm. Survival index and ratio of number of animals weaned per number of litters decreased at both 5,000 or 10,000 ppm. Dose-related decrease in weanling weights at all three dose levels.	--	Collins et al. 1971
Carbaryl (purity unspecified)	Gerbil (Mongolian)	Carbaryl was fed to gerbil at dietary levels of 0, 2,000, 4,000, 6,000, or 10,000 ppm for for three generations.	Reproductive effects at 6,000 or 10,000 ppm. Decreases in viability index, survival index, and average number of animals weaned/litter at 6,000 or 10,000 ppm.	--	Collins et al. 1971
Carbaryl (purity unspecified)	Rat (Harlan Wistar)	Six pregnant rats were given 0, 20, 100, or 500 mg/kg in the diet from pregnancy through weaning.	Not teratogenic.	--	Weil et al. 1972
Carbaryl (purity unspecified)	Monkey	Daily gastric intubation of carbaryl at 2 or 20 mg/kg	Higher abortion rate in treated than in control group. Examination of live infants and some aborted fetuses showed no teratogenic activity.	--	Dougherty et al. 1971

Appendix E (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	Reference
			Reproduction and Teratogenic Effects (Concluded)		
Carbaryl (99.56% pure)	Guinea pig (100 days of age)	Daily administration of carbaryl at either 300 mg/kg in diet or 200 mg/kg by oral intubation for three generations.	Not teratogenic.	--	Weil et al. 1973
Carbaryl (purity unspecified)	Guinea pigs	Guinea pigs given 300 mg/kg carbaryl orally in gelatin capsule daily during organogenesis (days 12-20 of gestation).	Teratogenic (skeletal defects mostly in cervical vertebrae).	This multiple treatment increased maternal deaths and fetal mortality.	Robens 1969
Carbaryl (purity unspecified)	Rabbit (New Zealand)	Rabbits given carbaryl 50 to 200 mg/kg orally in gelatin capsule daily from days 5-15 of gestation.	Not teratogenic at all doses.	--	Robens 1969
Carbaryl (99% pure)	Rabbit (New Zealand)	Rabbits were given 150, or 200 mg/kg in cotton seed oil by gavage from days 6-18 of gestation (200 mg/kg was a maternally toxic dose).	Teratogenic at both doses. Significant increase in incidence of omphalocele among offspring of rabbits given 200 mg/kg and one case was observed at 150 mg/kg.	--	Murray et al. 1979

Appendix E (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	Reference
			Carcinogenicity		
Carbaryl (97.65% pure)	Rat (Mongrel)	Forty-eight male rats treated by a subcutaneous implantation of paraffin capsule containing 20 mg of carbaryl. The control consisted of 48 male rats. These animals were kept for 22 mos.	Two of the ten treated rats surviving for 22 mos had fibrosarcomas at unspecified locations. In the control group, one of the 46 survived control had a fibrosarcoma at an unspecified location.	Authors concluded that carbaryl could produce tumors in rats. Results of these studies are not conclusive because of high mortality in treatment group and absence of information on how the controls were treated. In addition, not clear whether 2-naphthyl N-methylcarbamate (2-naphthyl isomer of carbaryl) was present in test substance. This isomer has been reported to have caused cancerous tumors in rats and in mice given orally or intravenously.	Andrianova and Alekseev 1970, in U.S. Department of Health, Education and Welfare 1976
Carbaryl (technical)	Rat (Mongrel)	Sixty male rats were given carbaryl at 30 mg/kg twice a wk for 22 mos (whether by incorporation in diet or by intubation not clear).	Examination of 12 survivors (survival rate was unspecified) of treatment group showed 3 fibrosarcomas and 1 fibrosarcoma occurred among 46 survived controls (statistically different ($p > 0.01$) from the control group.	Results of these studies are not conclusive because of the high mortality in the treatment group and the absence of information on how the controls were treated. Also not	Andrianova and Alekseev 1970, in U.S. Department of Health, Education and Welfare 1976

Appendix E (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	Reference
			Carcinogenicity (Continued		
Carbaryl (97.65% pure) (Cont'd)				clear whether 2-naphthyl isomer of carbaryl was present in test substance. This isomer has been reported to have caused cancerous tumors in rats and in mice given orally or intravenously.	
Carbaryl (purity unspecified)	Rat (male and female, (CF-N, male and female)	Groups of 20 rats of each sex were fed carbaryl in diets at 0, 2, 4, 8, or 16 mg/kg for 732 to 736 days.	Tumor incidences (tumor types unspecified) of treatment groups was not significantly different from controls.	--	Carpenter et al. 1961
Carbaryl (purity unspecified)	Rat (pregnant female)	Carbaryl (total dose of 300 mg over a 10-day period) was administered by gavage to pregnant rats with and without a 4% sodium nitrite solution.	Carbaryl alone or carbaryl and sodium nitrite together resulted in no significant incidence of malignant tumors when compared to historical controls.	There were no control groups.	Lijinsky and Taylor 1977, in Sternberg 1979

Appendix E (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	Reference
		Carcinogenicity (Continued			
Carbaryl (technical)	Mouse (male and female, (C57BL/6 x C3H/Anf)F_1 or C57BL/6 x AKR)F_1)	Groups of 18 mice of both sex and strain were administered 4.64 mg/kg carbaryl in gelatin by stomach tube daily from 7 days of age up to 4 wks of age. Subsequently, the mice were given 14 mg/kg until they were about 78 wks old. Groups of control mice (number unspecified) of each sex and strain were either untreated or received gelatin only.	Tumor incidences among surviving mice in the four groups did not differ statistically from 79 to 90 control mice in any sex-strain subgroups or in the combined sexes of either strain.	--	Innes et al. 1969 in U.S. Department of Health, Education, and Welfare 1976 in International Agency for Research on Cancer 1976

Appendix E (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	Reference
			Carcinogenicity (Continued		
Carbaryl (technical)	Mouse (male and female, C57BL/6 x C3H/Anf)F_1 or C57BL/6 x AKR)F_1)	Groups of 18 mice of both sex and strain were administered single subcutaneous injections of 100 mg/kg carbaryl in dimethyl sulfoxide from 4 wks to 78 wks of age. Groups of control mice (number unspecified) of each sex and strain were either untreated or given vehicle.	Tumor incidences in surviving mice (15, 16, 18, and 17) in the four groups were not statistically significant from 145 to 161 control mice in any sex-strain subgroups or in the combined sexes of either strain.	International Agency for Research on Cancer working group noted that a negative result after a single subcutaneous injection may not be adequate basis for discounting carcinogenicity.	Innes et al. 1969 in U.S. Department of Health, Education and Welfare, 1976, in International Agency for Research on Cancer 1976
Carbaryl (unspecified)	Mouse (male, A/JAX or C3H)	Groups of 30 3-mo-old mice were given weekly subcutaneous injections of carbaryl at 10 mg/animal (about 400 mg/kg/wk in a 25-g mouse) in 0.2 ml agar suspension over 5 mos.	Lung tumor incidences among treatment groups was not significantly different from controls.	--	Carpenter et al. 1961

Appendix E (Concluded)

Formulation	Organism	Nature of Exposure	Effects	Comments	Reference
		Carcinogenicity (Concluded			
Carbaryl (technical) (Cont'd)		Control mice (number unspecified) were either untreated or had 0.25% agar solution only.			
Carbaryl (unspecified)	Mouse (male, A/He)	Sixteen 7- to 9-wk old-mice given 12 intraperitoneal injections of carbaryl in tricaprylin over 4 wks. Total dose of carbaryl, 6 mg/animal. Three control groups of mice (32/group) were injected with water or vehicle tricaprylin or were untreated. The animals were kept 20 wks after last injection. The lungs were examined at autopsy microscopically for tumors.	Lung tumor incidences among treatment groups was classified as marginal.	Difficult to draw any conclusions from this study. From the structural formula given, it is not clear whether the test compound was 2-naphthyl isomer of carbaryl or carbaryl.	Shimkin et al. 1969, in U.S. Department of Health, Education, and Welfare 1978

Appendix E-1

Summary of N-Nitrosocarbaryl Toxicity Data for Mammals

Appendix E-1

Summary of N-Nitrosocarbaryl Toxicity Data for Mammals

Formulation	Organism	Dose	Comments	Reference
N-nitrosocarbaryl (purity unspecified)	Rat (Wistar, male and female)	Eight animals of each sex received single subcutaneous injection of N-nitrosocarbaryl suspended in Livio oil at 1 g/kg. Control groups (8 animals of each sex) received only Livio oil.	N-nitrosocarbaryl induced local sarcoma at the site of injection. By day 450, 14 of 16 rats died.	Eisenbrand et al. 1975
N-nitrosocarbaryl (percent purity unspecified)	Rat (Sprague-Dawley male and female, 8 to 10 wks old	Fifteen male rats were administered by gavage of N-nitrosocarbaryl twice a week for 20 wks (total dose of 300 mg). Twelve females rats were administered by gavage of N-nitrosocarbaryl once a week for 10 wks (total dose of 50 mg). These animals were observed for 120 wks.	Incidence of squamous stomach tumors in males and females were 47 and 75%. Males died earlier than females.	Lijinsky and Taylor 1976
N-nitrosocarbaryl, (purity unspecified)	Rat (Wistar, male and female, 3 mos old)	Thirty-seven rats of both sexes were given single oral doses of N-nitrosocarbaryl suspended in starch paste by stomach tube at 200 to 1,500 mg/kg. Twenty-two untreated male and female rats served as controls.	No tumors after 21 mos of treatment.	Eisenbrand et al. 1975

Appendix F

Summary of Carbaryl Neurotoxicity Data

Appendix F

Summary of Carbaryl Neurotoxicity Data

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl (99.9% pure)	Rat (Sprague Dawley, male)	Each of 6 rats was given intraperitoneal (ip) and intramuscular (im) injections of 1, 3, 5, or 10 mg/kg carbaryl 1 or 2 times/wk. Each rats served as its own control.	6 mos	Significant decreases in performance (response rate) after 3, 5, or 10 mg/kg ip and 5 or 10 mg/kg im injections of carbaryl.	--	Anger and Wilson 1980
Carbaryl (purity unspecified)	Rat	Dietary feeding of carbaryl at 100, 500, or 2,500 ppm.	35 days	Progressive decrease in the animals' ability to avoid electric punishment.	--	Tachibana et al. 1973 cited in Kuhr and Dorough 1976
Carbaryl (purity unspecified)	Rat (Wistar, male)	Dietary feeding of carbaryl at 100 or 200 ppm (equivalent to 10 or 20 mg/kg). Eight animals each were used for control and for the 2 treatment groups.	50 days	Carbaryl affected: 1. Process of learning in 200-ppm group and performance of a previously learned task in both groups after 50 days treatment. 2. EEG patterns (increase in fast and slow wave components) under resting and light-stimulated conditions in both groups.	--	Desi et al. 1974

Appendix F (Concluded)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Reference
Carbaryl (purity unspecified) (Cont'd)				3. Inhibition of AChE activity in various parts of the brain in 200 ppm group. 4. Mild, but permanent and increasing, functional deviations of the central nervous system.		
Carbaryl (purity unspecified)	Monkey (rhesus)	Route of administration and doses of carbaryl unspecified.	Not specified.	Carbaryl increased both the number of errors and the time required to finish a session in a repeated acquisition task.	—	Anger and Setzer 1979, in Miller 1982
Carbaryl (purity unspecified)	Swine	Single oral administration of carbaryl at 1 or 2 g/kg.	Not specified.	Salivation, central-nervous system depression, muscle tremors, and cyanosis.	—	Smalley et al. 1969 and Smalley 1970, in Kuhr and Dorough 1976
Carbaryl (purity unspecified)	Swine (13-wk old)	Dietary feeding of carbaryl at 150 to 300 ppm per day.	Up to 12 wks	Progressive myasthenia incoordination, ataxia, and clonic muscular contractions. Continued exposure resulted in paraplegia and prostration.	—	Smalley et al. 1969 and Smalley 1970, in Kuhr and Dorough 1976

Appendix G

Summary of Carbaryl Mutagenicity Data

Appendix G

Summary of Carbaryl Mutagenicity Data

Formulation	Organism	Nature of Exposure	Effects	Comments	Reference
Carbaryl (purity unspecified)	Barley (Hordeum vulgare	Sprouted barley seeds were treated with carbaryl at 1,000 ppm for 12 hrs.	Carbaryl induced chromosomal aberrations of 0.55% and 2.9% in the C-1 and C-2 generations. The untreated control sprouted seeds showed chromosomal aberrations of 0.22 and 0.49% in the C-1 and C-2 generations, respectively.	--	Wuu and Grant 1967
Carbaryl (84% pure)	Human embryonic fibroblasts	Treated human embryonic fibroblasts with 84% pure carbaryl at 20- to 80 μg/ml culture medium.	Mitosis and spindle fibre formation was inhibited by carbaryl.	--	Vasilos et al. 1972, in International Agency for Research on Cancer 1976
Carbaryl (purity unspecified)	Salmonella typhimurium LT 12	Carbaryl (concentration unspecified) was tested with a number of histidine auxotrophs of S. typhimurium.	Not mutagenic.	--	Regan et al. 1976, in Sternberg 1979
Carbaryl (purity unspecified)	S. typhimurium TA98, TA100, TA1535, or TA1537 strains	Concentrations of test substance unspecified. Test organisms treated with carbaryl in the presence of a rat-liver microsome induced by Arochlor.	Not mutagenic.	--	McCann et al. 1975, in International Agency for Research on Cancer 1976
Carbaryl (purity unspecified)	Escherichia coli	Test condition unspecified.	Not mutagenic (no reverse mutation).	--	Ashwood-Smith et al. 1972, in International Agency for Research on Cancer 1976

Appendix G (Continued

Formulation	Organism	Nature of Exposure	Effects	Comments	Reference
Carbaryl (purity unspecified)	*Saccharomyces cerevisiae*	Test condition unspecified.	Not mutagenic (no mitotic gene conversion).	--	Siebert and Eisenbrand 1974, in International Agency for Research on Cancer(1976
Carbaryl (85% pure)	*Drosophila melanogaster*	Test condition unspecified.	Mutagenic (increased number of recessive lethals).	--	Brzeskij and Veskov 1971, in International Agency for Research on Cancer 1976
Carbaryl (purity unspecified)	Rat (10 male)	Dominant lethal tests were conducted by dietary feeding of carbaryl up to 200 mg/kg/day or oral intubation of carbaryl up to 100 mg/kg/day until 32 wks of age.	No dominant lethal mutagenicity.		Weil et al. 1973
Carbaryl (purity unspecified)	Mouse	Ten male mice each received daily doses of carbaryl at 50 or 1,000 mg/kg for 5 days. One mouse in the group dosed at a rate of 1,000 mg/kg died. Each of 19 surviving males was caged for mating with 3 untreated virgins each week for 8 wks. The females	No dominant lethal mutagenicity.		Epstein et al. 1972

Appendix G (Concluded)

Formulation	Organism	Nature of Exposure	Effects	Comments	Reference
Carbaryl (purity unspecified) (Cont'd)		were subsequently killed on about day 13 of gestation and the total embryo implants in females mated with treated males and in females mated with untreated control males were compared.			
Carbaryl (purity unspecified)	Mouse (Swiss albino, male)	*In vivo* cytogenetic testing. Mice were treated orally with 146 mg/kg carbaryl. Each animal received the dose in two equal installments 24 hrs apart. Six hrs after the sec dose, animals were were killed and bone marrow removed for micronucleus test.	No significant increase in the frequency of micronuclei in the treated group when compared to control group.	--	Usha Rani et al. 1980
Carbaryl (purity unspecified)	Mouse (Swiss albino, male)	Host-mediated assay in which male Swiss albino mice given 483 mg/kg carbaryl orally. Doses given in 3 equal installments for 3 days. One day following the last treatment, the mice were injected intraperitoneally with *S. typhimurium* G46.	No significant increase in the frequency of revertants in the carbaryl treated animals.	--	Usha Rani et al. 1980

Appendix G-1

Summary of Mutagenicity Studies for N-Nitrosocarbaryl

Appendix G-1

Summary of Mutagenicity Studies for N-Nitrosocarbaryl

Formulation	Organism	Dose	Comments	Reference
N-nitroso-carbaryl (purity unspecified)	*Salmonella typhimurium* strains (TA98, TA100, TA1535, TA1537, and TA1538)	Dose of N-nitrosocarbaryl used was 0.00115, 0.0115, 0.115, 1.15, or 11.5 μg/plate.	N-nitrosocarbaryl mutagenic in both spot and plate incorporation tests.	Blevins et al. 1977
N-nitroso-carbaryl (purity unspecified)	*Saccharomyces cerevisiae*	0, 1, 5, 10, 25, or 50 ppm N-nitrosocarbaryl.	Induced mitotic gene conversions.	Siebert and Eisenbrand 1974

Appendix H

Summary of Metabolism Studies with Carbaryl

Appendix H

Summary of Metabolism Studies with Carbaryl

Formulation	Organism	Dose	Comments	Reference
		Absorption		
Carbaryl (^{14}C-naphthyl)	Rat (male)	Solution of carbaryl in acetone applied to shaved rats at the rate of 4 μg/cm^2. Radioactivity in urine measured for 5 days after treatment.	At 4 hours after dose 45.7% of the applied dose penetrated. A significant amount (95.7%) penetrated within 5 days.	Shah and Guthrie 1983
Carbaryl (^{14}C-methyl 99% pure)	Rabbit (female)	3.4 ml of acetone containing 3.4 mg of nonradioactive carbaryl and 68 μCi of ^{14}C-methyl labeled carbaryl was applied over a 2-cm^2 area of previously shaved skin. Blood sample was obtained by venipuncture from ear veins at 1, 5, 15, 30 min, and 1, 2, 3, 8, and 24 hr after treatment.	Radioactivity as carbaryl equivalent in the blood of rabbit maximized (73.3 ppm) 8 hrs after dose and declined slightly (49.8 ppm) after 24 hrs.	Shah and Guthrie 1977
Carbaryl (^{14}C-labeled, position of label unspecified)	Man	Radioactively labeled carbaryl applied on the ventral forearm of man.	Significant amount (73.9%) of the applied dose absorbed after 5 days of application. Maximum absorption rate 3% of the dose/hr.	Feldman and Maibach 1974
Carbaryl (^{14}C-labeled, position of label unspecified)	Man	Radioactively labeled carbaryl was applied on the jaw angle of man.	Significant amount (69.9%) of the applied dose was absorbed after 5 days of application. Maximum absorption rate not reported.	Maibach et al. 1971
Carbaryl (^{14}C-carbonyl)	Rat	Oral administration of ^{14}C-carbonyl-labeled carbaryl in rats.	Radioactive CO_2 in the exhaled air reached its peak 30- to 40 min after oral administration of ^{14}C-carbonyl-carbaryl showing the rapid absorption of carbaryl by gastrointestinal tract.	Casper and Pekas 1971, in Kuhr and Dorough 1976

Appendix H (Continued)

Formulation	Organism	Dose	Comments	Reference
		Absorption (Cont'd)		
Carbaryl (^{14}C-labeled, position of of label unspecified)	Rat (male)	Solutions of carbaryl (0.005 to 0.1 mM) administered into the lungs or small intestine of anesthesized rats. The organs were removed at various times and assayed for amount of carbaryl remaining.	Percentage of unabsorbed dose was plotted against time, and half-times (t 1/2) of absorption of carbaryl from the lungs and small intestine were calculated. Results showed that t 1/2 of absorption of carbaryl from the lungs and small intestine were 2.6 and 6.4 min. In addition, absorption of carbaryl by these organs per unit time was directly proportional to the dose of carbaryl administered.	Hwang and Schanker 1974
Carbaryl (99.9% pure)	Rabbit (male)	Isolated perfused lung dosed with carbaryl concs of 20, 40, 80, and 120 μg/ml perfusate. 5-ml serial samples of perfusate analyzed at 1, 5, 10, 15, 60 and 120 min.	Lungs absorbed 26% of the applied dose in 15 min of perfusion and uptake of carbaryl by the lungs was slow after this time. No evidence of saturation of uptake of carbaryl over the range of doses used.	Blase and Loomis 1976
Carbaryl (^{14}C-labeled, position of label unspecified)	Rat	Intratracheal injection of small volume (unspecified) of ^{14}C-labeled carbaryl.	Radioactivity in the blood of rats reached maximum in 2- to 5 min.	Nye and Dorough 1976
Carbaryl (purity unspecified)	Fish (Pseudorasbora parava)	Freshwater fish reared in water tank containing 1 ppm carbaryl for 30 days at water temp. of 23 ± 2 °C. The pH of water was 6.4 to 6.8. Carbaryl in fish was analyzed at various intervals for 1 mo.	Maximum level of carbaryl in fish reached 7.5 ppm after 1 day of exposure and decreased to 0.89 ppm after 7 days of exposure.	Kanazawa 1975

Appendix H (Continued)

Formulation	Organism	Dose	Comments	Reference
		Absorption (Cont'd)		
Carbaryl (99% technical, ^{14}C-naphthyl)	Catfish	Channel catfish were exposed to mixtures of ^{14}C-naphthyl-labeled carbaryl and non-radioactive carbaryl for 56 days continuously at the following doses: 0.05 mg/l or 0.25 mg/l in water and 0.28 mg/kg or 2.8 mg/kg/wk in kiet. Five fish were removed and analyzed after 3, 7, 14, 28, and 56 days of exposure. Previously exposed fish (0.25 mg/l in the water and 2.8 mg/kg/wk in diet) were placed on carbaryl-free diet for 0, 3, 8, 14, and 28 days to study retention of carbaryl in fish.	Total residues of carbaryl and metabolites accumulated by channel catfish during exposure for 56 days to 2.8 and 0.28 mg/kg/wk in feed was 9 ng and 1 ng/g body weight. Total residues accumulated by catfish during exposure for 56 days to 0.25 and 0.05 mg/l in water bath was 11 and 2 ng/g body weight. This study shows dose-dependency for both feed and water exposures. When fish exposed to 2.8 mg/kg/wk in diet were placed on a carbaryl-free diet for 28 days, residue level reached 22% of the 0-day residue level Residues remained constant for 28 days in fish previously exposed to carbaryl at 0.25 mg/l in their water bath.	Korn 1973
Carbaryl (^{14}C-naphthyl, 99% pure)	Rainbow trout	Ten rainbow trout were placed in a 75-l tank containing 55 l of continuously aerated water and kept at 12 °C. Carbaryl conc. in the tank was 0.25 mg/l.	Approximately 15% of the applied carbaryl in tank water was absorbed by trout in 24 hrs. Radioactivity in bile represented 30% of the total quantity of ^{14}C-carbaryl taken up by the fish.	Statham et al. 1975
Carbaryl (^{14}C-naphthyl)	Algae, clam, crab, *Daphnia*, *Elodea*, mosquito fish (*Gambusia affinis*), mosquito, and snail	Unspecified	All the organisms contained substantial radioactivity. Conc. of ^{14}C-carbaryl and its metabolites in these organisms ranged between 0.428 and 5.13 ppm. Thin layer chromatographic analysis of each organism showed few radioactive spots, whose, identities were unknown. None of the organisms contained carbaryl.	Sanborn 1974

Appendix H (Continued)

Formulation	Organism	Dose	Comments	Reference
Absorption (Cont'd)				
Carbaryl (^{14}C-naphthyl)	House fly, stable fly, boll weevil, and rice weevil	Flies were treated with 2 μg/fly on ventral portion of the abdomen and weevils were treated with 5 μg/weevil on dorsal surface of prothorax.	Topical carbaryl dose absorbed by each species of insect within 24 hrs was: stable fly, 56.7%; house fly, 37.3%; boll weevil, 12.2%; and rice weevil, 3.3%.	Camp and Arthur 1967
Carbaryl (99.85% pure)	German cockroach (Blattella germanica)	1.67 μl of acetone containing 4 or 16 μg of carbaryl was applied to resistant, and susceptible strains. Internal tissues and feces were collected at various times (at 6- and at 12-hr intervals up to 108 hrs) after carbaryl treatment.	Rate of penetration was based on total amounts of carbaryl, 1-naphthol, and conjugates of 1-naphthol recovered from tissues and excreta. Susceptible and resistant strains absorbed 51.3 and 48.1% of the applied dose during the first 36 hours after treatment, but thereafter penetration was about equal.	Ku and Bishop 1967
Carbaryl (^{14}C-naphthyl, 98% pure)	Gypsy moth (Lymantria dispar)	Larvae of gypsy moth were dosed topically with ^{14}C-naphthyl-labeled carbaryl and penetration of carbaryl was followed for 12 hrs.	Penetration rates of carbaryl during 0 to 5 mins, 5 to 60-mins, 1 to 3 hrs, and 3 to 12 hrs were 2.8, 0.57, 0.07, and 0.02% min.	Ahmad et al. 1980
Distribution				
Carbaryl (^{14}C-naphthyl, ^{14}C-carbonyl, ^{14}C-methyl)	Rat	Four rats dosed orally with ^{14}C-labeled carbaryl at 20 mg/kg were sacrificed at 7 days after dose and their tissues were analyzed for ^{14}C.	Neither ^{14}C-naphthyl nor ^{14}C-carbonyl residues could be detected. Radioactivity in intestinal tracts, carcasses, and remaining organs (unspecified) of rats treated with ^{14}C-methyl carbaryl amounted to 2 to 3% of the administered dose.	Knaak et al. 1965

Appendix H (Continued)

Formulation	Organism	Dose	Comments	Reference
Distribution (Cont'd)				
Carbaryl (^{14}C-naphthyl)	Rat (male)	4 µg/cm^2 carbaryl was applied to shaved rats. Radioactivity was measured in various tissues at interv of 4, 8, 12, 24, 48, and 120 hrs after treatment.	Radioactivity in carcass decreased with time. Less than 0.1% of total dose was found in blood at any interval. Radioactivity in liver was 0.13% of total dose at 120 hrs post-treatment.	Shah and Guthrie 1983
Carbaryl (^{14}C-naphthyl	Rat (female)	Nonpregnant and pregnant rats at day 18 to 19 of gestation were injected intraperitoneally with a dose (unspecified) of either ^{14}C-naphthyl or ^{14}C-carbonyl-labeled carbaryl.	^{14}C declined rapidly for 1 to 2 hrs and declined at a slower rate thereafter. After 8 hrs, about 3% of the dose was in the fetus, showing that carbaryl crossed the pacenta. Fetal brain, heart, and lung contained more ^{14}C on a weight basis than maternal counterparts.	Strother and Wheeler 1976
Carbaryl (^{14}C-carbonyl	Chicken (mature Leghorn hen)	Three hens were dosed with ^{14}C-labeled carbaryl (5 to 10 µCi) at 10 mg/kg in a gelatin capsule. The hens were sacrificed at 48 hrs, and tissues were analyzed for ^{14}C.	Total radioactivity remaining in 7 organs (liver, kidney, gizzard, heart, intestine, proventriculus, and pancreas) and carcass 48 hrs after dosing accounted for 7.1% and 1.4% of the ^{14}C-carbonyl and ^{14}C-naphthyl carbaryl.	Paulson and Feil 1969
Carbaryl (^{14}C-naphthyl, 99% pure)	Chicken (mature Leghorn hen)	Hens were fed ^{14}C-naphthyl-labeled carbaryl (7, 21, and 70 ppm) for 14 days in diet.	Seven days after dosing, 0.153 and 0.05% of the dose (expressed as average of three dose levels) were recovered in eggs and carcass. Residues reached maximum within 2 days in egg white and 6 to 8 days in egg yolk. After discontinuation of	Andrawes et al. 1972

Appendix H (Continued)

Formulation	Organism	Dose	Comments	Reference
		Distribution (Cont'd)		
Carbaryl (^{14}C-naphthyl 99% pure) (Cont'd)			dosing half-life of residues was less than 1 day in the egg white, 2 to 3 days in egg yolk, and 5 days in carcass.	Andrawes et al. 1972
Carbaryl (purity unspecified)	Chicken (mature Leghorn hen)	Nine hens received daily oral dose of carbaryl at 0, 180, and 540 mg/kg body weight for 60 days.	At 180 mg/kg body weight, 1 to 2 ppm carbaryl equivalent appeared in fatty tissues on the 14th day of administration and this level did not increase in spite of the continued feeding of the carbaryl up to 60 days. At 540 mg carbaryl/kg body weight, residue levels no higher than 1 to 2 ppm carbaryl equivalent were found in muscle after 5 wks of administration. No detectable carbaryl or its naphthol metabolite could be found in liver, gizzard, or heart muscle of hens at either level of administration.	Nir et al. 1966
Carbaryl (80% WP)	Chicken (mature hen)	Hens were dipped in 0.5 or 1.0% water suspension of carbaryl.	Residues of carbaryl and two of its metabolites, 1-naphthol and and N-hydroxycarbaryl in eggs reached maximum 5 to 7 days after dipping. The levels of carbaryl, 1-naphthol, and N-hydroxycarbaryl were 0.08 to 0.14, 0.12 to 0.20, and 0.06 to 0.12 ppm. Residue levels in eggs were detectable 56 days post-treatment but did not exceed 0.5 ppm egg tolerance for carbaryl and its metabolites.	Ivey et al. 1983

Appendix H (Continued)

Formulation	Organism	Dose	Comments	Reference
		Distribution (Cont'd)		
Carbaryl (^{14}C-carbonyl labeled)	Cow	2 g of ^{14}C-carbonyl-labeled carbaryl was given orally to a lactating cow. Milk samples obtained at 9 and 10 hrs post-treatment were analyzed for ^{14}C.	Radioactive residue in milk was equivalent to 1% of the administered dose. 85% of radioactive residue consisted of lactose, a natural component of of milk.	Baron 1968
Carbaryl (purity unspecified)	Bee	Bees were fed 2 ppm carbaryl in honey solution and in sugar solutions for 56 days then carbaryl-free solutions.	At the end of 56 days, carbaryl residue found in honey, bee bread and bees ranged from 0.025 to 0.05 ppm, 0.04 to 0.08 ppm and 0.016 to 0.02 ppm. At the end of carbaryl feeding, detectable amounts of carbaryl residues in honey, bee bread, and and bees were found, even up to 64 days post-treatment. up to 64 days post-treatment.	Winterlin et al. 1973
Carbaryl (50% WP)	Fish (*Saccobranchus fossilis* and *Labeo vohita*)	Fishes were placed in the tank containing 8.42 mg carbaryl/ml (sublethal conc.). Quantitative estimation of carbaryl was made in the gills, intestine, liver, kidney, and muscles after the treatment for 4, 8, 15, 30, 45 days.	Accumulation of carbaryl was highest after treatment for 4 days as compared to after 8, 15, 30, and 45 days treatment. The amounts of carbaryl were highest in the gills (6.6 to 6.8 ppm) and least in the intestine (3.0 to 3.3 ppm) while the amount found in the liver, kidney, and muscles was between these two extremes. Carbaryl was found only in the gills (0.32 to 0.44 ppm) 45 days post-treatment.	Verma et al. 1977

Formulation	Organism	Dose	Comments	Reference
		Distribution (Cont'd)		
Carbaryl (^{14}C-naphthyl, 99.85% pure)	Catfish, crayfish, snail, algae and duckweed	Carbaryl was applied to an aquatic ecosystem consisting of 10 kg of Matapeake soil, 80 l of water and 5 organisms. All organisms were harvested after 20 days of exposure. Maximum carbaryl conc. in water was 16 ppb after 7 days and increased after 20 days.	Carbaryl conc. in the dried tissue of alage, duckweed, snail, crayfish, and catfish were 37.9, 34.2, 2.81, and 1.33 ppm.	Kanazawa et al. 1975
Carbaryl (purity unspecified)	Roe deer	Unspecified	Carbaryl residue in 70 brain, 80 lung, and 56 testis samples from field-living roe deer and 22 samples from the same organs taken from forest-living deer were analyzed. No perceptible carbaryl residue was found in any of the above organs from roe deer, the forest or field population.	Krynski et al. 1982

Formulation	Organism	Dose	% Excretion			Reference
		Excretion[a]	^{14}CO	Urine	Feces	
Carbaryl						
(^{14}C-naphthyl	Rat	8.5 to 26 mg/kg body weight	0	70	8	Knaak et al. 1965
^{14}C-methyl,	Rat		5	63	4	
^{14}C-carbonyl)	Rat		30	45	8	
Carbaryl						
(^{14}C-naphthyl	Pig	25 mg/kg body weight	—	68	2	Knaak et al. 1968
^{14}C-methyl)	Pig		—	56	1	
Carbaryl						
(^{14}C-naphthyl	Sheep	25 mg/kg body weight	—	70	3	Knaak et al. 1968
^{14}C-methyl)	Sheep		—	52	5	

Appendix H (Continued)

Formulation	Organism	Dose	Comments			Reference
		Excretion (Cont'd)	% Excretion			
			^{14}CO	Urine	Feces	
Carbaryl (^{14}C-naphthyl	Cow	0.25 and 3.05 mg/kg body weight	--	68	11	Dorough 1967
^{14}C-carbonyl)	Cow	2 g/cow	--	30	--	Baron et al. 1969
Carbaryl (^{14}C-naphthyl	Chicken	10 mg/kg body weight	0	94	7	Paulson and Feil 1969
^{14}C-carbonyl)	Chicken		49	31	6	
Carbaryl (^{14}C-carbonyl)	Gerbil Guinea pig Mouse	0.2 mg/kg body weight	65 58 26	18 10 26	5 4 6	Benson and Dorough 1984
Carbaryl (^{14}C-naphthyl	Dog	10 mg/kg body weight	--	30	15	Knaak and Sullivan 1967
^{14}C-carbonyl)	Dog		--	10	5	
Carbaryl (nonradioactive)	Man	2 mg/kg body weight	--	28	--	Knaak et al. 1968
Carbaryl (^{14}C-naphthyl)	Rat	Single oral dose of carbaryl (0.01 mg/kg) in 0.2 ml of vegetable oil administered by intubation to two bile-duct-cannulated rats. Bile, urine, and feces were analyzed for radioactivity.	Within 2 days, radioactivity in bile, urine, and feces accounted for 45.4, 42.3 and 1.4% of the administered dose, indicating that oral exposure to carbaryl resulted in enterohepatic circulation of carbaryl and its metabolites.			Marshall and Dorough 1979
Carbaryl (^{14}C-carbonyl)	Rat	^{14}C-carbonyl-labeled carbaryl (1.5, 30, and 300 mg/kg body weight) was given to bile-fistulated rats by oral administration.	From 15 to 46% of the administered radioactivity secreted in bile, 10 to 40% in urine, and less than 1% in feces 12 hrs after dosing of ^{14}C-carbonyl-labeled carbaryl.			Struble et al. 1983

Appendix H (Continued)

Formulation	Organism	Dose	Comments	Reference
		Excretion (Cont'd)		
Carbaryl (99.85%)	German cockroach (*Blattella germanica*)	1.67 μl of acetone containing 4 or 16 μg of carbaryl was applied to resistant and susceptible strains. Internal tissues and feces were collected at 6-hr and 12-hr intervals up to 108 hrs after carbaryl treatment.	Excretion of carbaryl and its metabolic products was more ranid in resistant strain than in susceptible strain. This appears to be the contributing factor in the difference of susceptibilities to carbaryl in these 2 strains.	Ku and Bishop 1967
		Biotransformation		
Carbaryl (^{14}C-naphthyl, ^{14}C-methyl, ^{14}C-carbonyl)	Rabbit	1 g/rabbit	The following metabolites of carbaryl were found in the urin of the indicated species: I. 1-naphthol	Leeling and Casida 1966
	Chicken	100 mg/kg body weight	I. 1-naphthol	Paulson et al. 1970
		Details not given.	II. methylcarbamic acid (unconfirmed)	Menzie 1978
	Rabbit	1 g/rabbit	III. 4-hydroxycarbaryl	Leeling and Casida 1966
	Chicken	100 mg/kg body weight	III. 4-hydroxycarbaryl	Paulson et al. 1970
	Rabbit	1 g/rabbit	IV. 5-hydroxycarbaryl	Leeling and Casida 1966
	Chicken	100 mg/kg body weight	IV. 5-hydroxycarbaryl	Paulson et al. 1970
	Rabbit	1 g/rabbit	V. 5,6-dihydro-5,6-dihydroxycarbaryl	Leeling and Casida 1966
	Rat	30 mg/kg body weight	V. 5,6-dihydro-5,6-dihydroxycarbaryl	Sullivan et al. 1972

Appendix H (Continued)

Formulation	Organism	Dose	Comments	Reference
		Biotransformation (Cont'd)		
		Details not given.	VI. 1,3,4-trihydroxynaphthalene	Menzie 1978
	Chicken	100 mg/kg body weight	VII. 1,5,6-trihydroxynaphthalene	Paulson et al. 1970
	Rabbit	1 g/rabbit	VIII. N-hydroxycarbaryl	Leeling and Casida 1966
		Details not given.	IX. 3,4-epoxide of carbaryl (unconfirmed)	Ryan 1971
		Details not given.	X. 5,6-epoxide of carbaryl (unconfirmed)	Ryan 1971
		Details not given.	XI. N-glucuronide of carbaryl (unconfirmed)	Menzie 1978
	Rat	0.1 to 1.0 mg/kg	XII. thioether amino acid conjugate of carbaryl (unconfirmed)	Bend et al. 1971
			All hydroxylated metabolites (I and III to VII) have been excreted primarily as water-soluble glucuronide or sulfate conjugates.	
Carbaryl (^{14}C-naphthyl, ^{14}C-carbonyl)	Rat	^{14}C-carbonyl-labeled carbaryl (30 and 300 mg/kg) and ^{14}C-naphthyl-labeled carbaryl (30 mg/kg) was given to bile-fistulated rats by oral administration.	Metabolites of carbaryl from bile identified by mass and/or NMR spectrometric methods were: 5,6-dihydro-5,6-dihydroxycarbaryl glucuronide, conjugates of carbaryl, and conjugated isomers of hydroxycarbaryl.	Struble et al. 1983
Carbaryl (^{14}C-naphthyl)	Rat	The cranial region of the small intestine caudal to the bile duct was removed, everted and incubated for 120 mins in Krebs-Ringer phosphate medium containing 10^{-5} M carbaryl.	Metabolites of carbaryl identified were as follows: hydroxycarbaryl, 1-naphthol, hydroxy-1-naphthol, dihydroxy-1-naphthol, dihydro-dihydroxy-1-naphthol and 1-naphthyl glucuronide, and hydroxy-1-naphthyl glucuronide.	Pekas 1979

Appendix H (Continued)

Formulation	Organism	Dose	Comments	Reference
		Biotransformation (Cont'd)		
Carbaryl (^{14}C-naphthyl)	Rat	Carbaryl (0.61 μmol rat) was administered into the small intestine. The portal blood was collected for 60 mins by use of a portal vein fistula and analyzed.	Principal metabolite in portal blood was naphthyl glucuronide.	Pekas 1983
Carbaryl (^{14}C-naphthyl 99.9%)	Rat Mouse Rabbit	Liver microsomes from rat, mouse, and rabbit were incubated with 40 mg of ^{14}C-carbaryl for 4 hrs at 37 °C.	The following metabolites were formed from carbaryl incubated with microsomes from rat, mouse, and rabbit livers: N-hydroxycarbaryl, 4-hydroxycarbaryl, 5-hydroxycarbaryl, 5,6-dihydro-5,6-dihydroxycarbaryl, and 1-naphthyl.	Leeling and Casida 1966
Carbaryl (^{14}C-naphthyl)	Human embryonic lung cell cultures	Lung cell cultures were incubated with ^{14}C-carbaryl for 72 hrs at conc. 300,000 to 400,000 ppm carbaryl.	Analysis of ^{14}C showed that 70% of recovered radioactivity resulted from oxidative metabolites, with 30% being water-soluble metabolites. [The oxidative metabolites based on a thin layer chromatographic analysis were 1-naphthol, 5-hydroxycarbaryl, 4-hydroxycarbaryl, and 5,6-dihydro-5,6-dihydroxycarbaryl.]	Lin et al. 1975
Carbaryl (^{14}C-methyl, ^{14}C-naphthyl) ^{14}C-naphthyl)	Mouse	Carbaryl (40 μg) was incubated with 2 ml of supernatant from the centrifugation at 10,000 g of mouse liver homogenate for 4 hours at 37 °C.	Mouse-liver preparation produced S-(4-hydroxy-1-naphthyl) cysteine and S-(5-hydroxy-1-naphthyl) cysteine. Identities were not confirmed.	Bend et al. 1971

Appendix H (Continued)

Formulation	Organism	Dose	Comments	Reference
		Biotransformation (Cont'd)		
Carbaryl (^{14}C-naphthyl, ^{14}C-methyl)	Rat Dog Guinea pig Man	2-mm cubes of liver tissue (0.5 g) were transferred to petri dish containing 3 ml of medium. Carbaryl (100 μg) was added and incubated for 18 hrs at 37 °C.	Medium containing the carbaryl metabolites was analyzed by column chromatography. In vitro results closely paralled in vivo me metabolism of the compound as derived from analysis of 24-hr urine urine samples. Metabolic products found in vitro were species-specif snecific and not dependent upon the addition of cofactors for their production.	Sullivan et al. 1972
Carbaryl (^{14}C-naphthyl, ^{14}(-carbonyl)	Fish (Trichogaster pectoralis)	Livers of fish (both sexes) were removed and microsomal fraction was prepared. Carbaryl was incubated (conc. unspecified) with microsomal fractions with or without NADPH$_2$ for 60 mins in the presence of air.	Metabolism study of carbaryl by the microsomal fraction of fish produced following metabolites. N-hydroxycarbaryl, 1-naphthol, and 5-hydroxy-carbaryl.	Gill and Yeoh 1980
Carbaryl (^{14}C-naphthyl, 99% pure)	Rainbow trout	Ten rainbow trout were placed in a 75-l tank containing 55 l of water, continuously aerated and kept at 12 °C. Carbaryl conc. in the tank was 0.25 mg/l.	Metabolites in bile were identified tentatively as 1-naphthyl glucuronide and 5,6-dihydro-5,6-dihydroxycarbaryl.	Statham et al. 1975

Appendix H (Continued)

Formulation	Organism	Dose	Comments	Reference
		Biotransformation (Cont'd)		
Carbaryl (99.85% pure)	German cockroach (*Blattella germanica*)	1.67 µl of acetone containing 4 or 16 µg of carbaryl was applied to resistant and susceptible strains. Internal tissues and feces were collected at 6- and at 12-hr intervals up to 108 hrs after the carbaryl treatment.	Carbaryl and 7 metabolites were detected in tissues or feces, although all metabolites did not appear after any one exposure period. Of the 7 metabolites, only 1-naphthol was positively identified. There were no qualitative differences in metabolites between the 2 strains in tissues or excreta.	Ku and Bishop 1967
Carbaryl (^{14}C-naphthyl and ^{14}C-carbonyl)	Tick	Adult female cattle ticks were injected through the ventral surface on the 4th day after dropping from the host animal. A dimethyl sulfoxide solution containing ^{14}C-labeled carbaryl (0.5 to 0.7 µg) in 1 to 3 µl was used for each tick.	Recovery of radiolabel in the aqueous acetone extract of ticks 48 hours after dosing with carbaryl was 90% for ^{14}C-naphthyl and 6% for ^{14}C-carbonyl, showing that the bulk of the carbamate ester bond of the injected carbaryl had been cleaved. The radioactivity in the acid hydrolyzed extract accounted for 1-naphthol (55%) and 1,5-hydroxynaphthalen (45%). No unchanged carbaryl could be detected.	Bend et al. 1970
Carbaryl (^{14}C-label, position of label unspecified)	Blowfly (*Calliphora erythrocephala*)	Various tissues of blowfly larvae were incubated with ^{14}C-carbaryl at 30 °C (ranges of carbaryl conc. were unspecified).	Carbaryl was most actively metabolized by the fat body. Metabolism of carbaryl was dependent on age of larvae from which fat bodies were isolated. A maximum rate of metabolism occurred with fat bodies from 4-day-old larvae. Rate decreased as larvae grew older.	Price and Kuhr 1968
Carbaryl (^{14}C-naphthyl, 98% pure)	Gypsy moth (*Lymantria dispar*)	Larvae of the gypsy moth were dosed topically with ^{14}C-naphthyl-labeled carbaryl and metabolism of carbaryl was followed for 12 hrs.	Ninety percent of the metabolism of carbaryl occurred within first 3 hrs. Over the next 9 hrs, metabolism was slow, with a linear rate of 0.8% hrs.	Ahmad et al. 1980

Appendix H (Concluded)

Formulation	Organism	Dose	Comments	Reference
		Biotransformation (Cont'd)		
Carbaryl (^{14}C-naphthyl, 98% pure) (Cont'd)			Unchanged carbaryl found in larvae and feces, were 16 and 9% of the applied dose at 12 hrs. The metabolic composition in the larvae at 12 hrs was 5-hydroxy carbaryl, N-hydroxycarbaryl, 1-naphthol, 4-hydroxycarbaryl, and 5,6-dihydro-5,6-dihydroxy-carbaryl.	
Carbaryl (^{14}C-naphthyl, 99% pure)	Boll weevil (*Anthonomus grandis*) bollworms (*Heliothis zea*)	Groups of 50 boll weevils, 3-5 days old, were treated topically on the dorsal prothoracic area with 1 µl of acetone containing 1 µg of carbaryl. Bollworm larvae and adults were injected with 1 µl of corn oil containing 1 and 0.5 µg of carbaryl, respectively.	In both species, carbaryl was metabolized hydrolytically oxidatively. Metabolites tentatively identified were 1-naphthol, 1-hydroxy-5,6-dihydro-5,6-dihydroxynaphthalene, 5,6-dihydro-5,6-dihydroxy-carbaryl, and N-hydroxycarbaryl.	Andrawes and Dorough 1967
Carbaryl (^{14}C-naphthyl, 98% pure)	Roach (*Blaberus disoidalis*) hornworm (*Manduca sexta*)	Carbaryl (0.1 µg) was applied to intact midgut tissues immersed in buffer.	Metabolism of carbaryl after 80 mins of treatment was slow; and little differences in metabolic patterns were noted between two species. Percent of radioactivity found as carbaryl and 1-naphthol in these species were 80 to 84 and 11 to 12%, respectively.	Shah and Guthrie 1971
Carbaryl (^{14}C-carbonyl, 98%)	Silkworm (*Bombyx mori*)	Two-day-old 5th-instar larvae and 7-day-old 5th-instar larvae were treated with carbaryl at 50 µg/g and 1.5 µg/g body weight.	Analysis of tissues showed that the primary metabolite of carbaryl was 2-hydroxycarbaryl.	Moriyama et al. 1972

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[a] ^{14}C-carbaryl equivalents expressed as percent of orally administered dose after 24 hours.

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Insecticide Background Statement: Diflubenzuron

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Diflubenzuron Summary

Major Trade Names: Dimilin®, Micromite®, Vigilante®, Largon®

Chemical Names: N-[[(4-chlorophenyl)amino]carbonyl]-2,6-difluorobenzamide. Also known as diflubenzuron, (DFB), dimethylsulfoxide, (DMSO), trichloroacetic acid, (TCA), TH 6040, PH 6040, OMS 1804, ENT-29054

Major Forestry Applications: Applied for insect pest control (gypsy moth, Douglas-fir tussock moth, forest tent caterpillar, Nantucket pine tipmoth) to woodland trees and shrubs including Christmas trees. Also applied to agricultural crops (cotton and soybeans), nurseries (ornamentals), pasture (California flood-irrigated areas only), and greenhouses (mushrooms).

Toxicity

Diflubenzuron is an insect growth regulator (IGR) that functions by inhibiting chitin synthesis during molting stages of the insect pest's/life cycle. The compound is toxic to a larger number of insects, but relatively nontoxic to fish, mammals, and birds.

Environmental Fate

Diflubenzuron is readily degradable in the environment and does not persist in soil or water. Diflubenzuron exhibits virtually no mobility either in the soil or from treated soils to plants. Although some residues persist on plant surfaces, the residues decline through dilution by growth of the plant. Diflubenzuron does not photolytically degrade on nonbiological surfaces.

Diflubenzuron has a short half-life of 0.5 to 1 week in soil, which is apparently unrelated to soil type but rather dependent on the microbial activity of the soil and the particle size of diflubenzuron applied (Verloop and Ferrel 1977, Seuferer et al. 1979).

Diflubenzuron degrades rapidly in neutral or alkaline waters. Its half-life in natural waters (pH 6 to 9) is less than 1 week. The compound has the potential for persistence or accumulation in water only under quite acidic conditions (Ivie et al. 1980). According to the literature, bioaccumulation of diflubenzuron in aquatic organisms during exposure generally does not exceed 100 times over ambient levels, and is reversible after removal of exposure.

Studies on the fate of diflubenzuron in air have not been reported.

1.0 General Information

Diflubenzuron is also known by the trade names Dimilin®, Micromite®, Vigilante®, and Largon® and as TH 6040 and PH 6040. The benzoylphenylurea compound was discovered by scientists at Philips-Duphar, B.V., The Netherlands and was developed by the Thompson-Hayward Chemical Company for market in the United States. Diflubenzuron is currently marketed in the U.S. by Uniroyal Chemical Corporation.

A wide variety of insect pests are controlled by diflubenzuron including the gypsy moth, certain beetles, flies, mosquitoes, gnats, rust mites, and weevils. Diflubenzuron is especially effective against most "worms" (i.e., caterpillars of moths and butterflies) that feed on foliage. Diflubenzuron has greatly reduced the population of gypsy moth larvae and protected against defoliation as a result of field trials in several states. The most extensive studies of diflubenzuron in the field were done in conjunction with the expanded Gypsy Moth Research and Application Program of the Forest Service.

Diflubenzuron is an insect growth regulator that inhibits chitin synthesis in insect pests. Chitin, a polysaccharide, is a major component in the thick cuticle covering of insects. The inhibition of chitin synthesis during molting kills insect pests and serves to prevent reproduction. The compound is not lethal for insects that have already reached the adult stage. Current studies indicate that diflubenzuron has relatively few adverse effects on nontarget species. Possible exceptions may include some microorganisms, aquatic insects, and small crustaceans that also synthesize chitin. Diflubenzuron generally has only short-term effects against sensitive nontargets and is not persistent in habitats where it would be applied.

1.1 Normal Use Patterns

Three formulations of diflubenzuron are now commercially available: a 25 percent wettable powder, a 45 percent oil-dispersible flowable concentrate, and a granule. Formulations of diflubenzuron are described in table 1-1. Diflubenzuron in the 25 percent wettable powder or oil-based formulation is used as a foliar spray (aerial or ground), or incorporated into the growing medium for mushrooms. Granules are used in field formulations for mosquito control.

In forestry applications, diflubenzuron is used primarily for gypsy moth eradication. The application rate for specific USDA Forest Service projects in 1985 was 0.01 and 0.06 pounds active ingredient per acre (lb a.i./acre) per treatment. Application of the compound is via aerial or ground spray of either a.i. aqueous Dimilin or oil Dimilin. Data on use of diflubenzuron by the USDA Forest Service are presented in table 1-2.

Table 1-1

Diflubenzuron Formulations

Formulation Active Ingredient	Formulator
Diflubenzuron (TH 6040) 25% wettable powder Technical crystalline	Thompson-Hayward Chemical Company Kansas City, MO
Diflubenzuron (TH 6040)	Philips Duphar, The Netherlands (Duphar, B.V. Amsterdam)
Diflubenzuron	Marketed by Uniroyal Chemical Company in U.S.
Dimilin WP-25 wettable powder 25% a.i.	Marketed by Uniroyal Chemical Company in U.S.
Dimilin G-0.5 granular 0.5% a.i.	Marketed by Uniroyal Chemical Company in U.S.
Dimilin G-4 granular 4% a.i.	Marketed by Uniroyal Chemical Company in U.S.
Dimilin ODC-45 450 g a.i./l	Marketed by Uniroyal Chemical Company in U.S.
Dimilin DP dustable powder	Marketed by Uniroyal Chemical Company in U.S.

Table 1-2

USDA Forest Service Use of Diflubenzuron

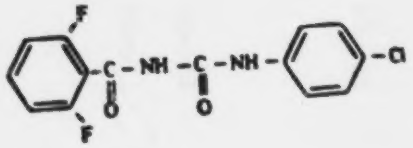
Purpose	Active Ingredient/Acre (lbs)		Acres Treated	Formulation	Application Method
	Range	Average			
1983					
Research (Pandora Moth)	NA	0.06	50	Liquid	Aerial
1984	0	0	0	--	--
1985					
Gypsy Moth	--	0.01	8991	Liquid	Ground
Gypsy Moth	--	0.06	3000	Liquid	Aerial
1986	0	0	0	--	--
1987					
Gypsy Moth	--	0.03	4654	Liquid	Aerial (helicopter)

NA - data not available.

Source: Gross 1987.

1.2 Physical and Chemical Properties

The active ingredient of diflubenzuron formulations is N-[[(4-chlorophenyl)amino]carbonyl]-2,6-difluorobenzamide. Other names include Dimilin, Vigilante, difluron, diflubenzon, diflubenuron, Largon, DV 112307, OMS 1804, PH 6040, TH 6040, TH 6040 and 1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl) urea. Diflubenzuron has the following molecular structure:



The compound is synthesized by the reaction of 2,6-difluorobenzamide with p-chlorophenylisocyanate, and has the molecular formula, $C_{14}H_9ClF_2N_2O_2$.

Technical diflubenzuron (i.e., at least 95 percent pure material) is a white to off-white crystalline solid, is odorless, and has a melting point between 210 and 230 °C. Vapor pressure is less than 1.3×10^{-5} Pa at 50 °C. The density/specific gravity is 1.2089 g/cm^3. Diflubenzuron is soluble in water (0.1 mg/l at 20 to 25 °C) and most organic solvents. Solubility in a polar solvents (ether, benzene, petroleum ether) is poor. It has an octanol/water partition coefficient of about 5,000. The log P (octanol/water) coefficient is 3.89. Diflubenzuron is stable at low pH in sterile water but is less stable at higher pH levels, particularly at higher temperatures. It is stable in ultraviolet light.

2.0 Toxicity of Diflubenzuron

Diflubenzuron is an insect growth regulator that is toxic to some invertebrates, but not to fish, birds, and mammals. Current data indicate that diflubenzuron is neither mutagenic nor carcinogenic, even at high dose levels. No teratogenic effects have been demonstrated in developing mice and no significant malformations are found in developing chicken embryos exposed to high levels of diflubenzuron.

The toxic effects of diflubenzuron results from its inhibition of chitin synthesis in the target pests for which it was developed. Inhibition of chitin synthesis during the molting process of insect development results in death of the insect. Table 2-1 summarizes the toxicity data for diflubenzuron from various studies.

Many studies of diflubenzuron toxicity deal with effectiveness of the compound in the control of insect pests. The results of some of these studies on target organisms are briefly summarized in table 2-2 and will not be considered further in this pesticide background statement.

In the following discussion of diflubenzuron toxicity, mutagenicity tests and studies are included in the section on mammalian toxicity, regardless of the specific test system or organism used because the ultimate purpose of such mutagenicity studies is to determine the potential mutagenicity activity of the test compound in humans.

2.1 Microorganisms

Appendix A summarizes the available data on the toxic effects of diflubenzuron on nontarget soil microorganisms and on aquatic algae. Although the available data base is not as extensive as for some other pesticides, results indicate decreases in populations of some soil-inhabiting microorganisms after application of diflubenzuron.

Seuferer et al. (1979) report on the metabolism of diflubenzuron by four species of soil fungi and the mutagenicity of the metabolites detected by a Salmonella mutagenicity test. Results of the laboratory study indicate that diflubenzuron was rapidly broken down by the four species of eukaryotic microorganisms examined, and the authors conclude similar degradation of the compound would occur by other eukaryotic species. Although one of the metabolites, 2,6-difluorobenzoic acid, was found to be lethal to soil microorganism (i.e., Salmonella), the author doubts that concentrations of this compound in the soil would result in pronounced toxicity.

Hanson and Garton (1982a) reported on effects to the total biological community in complex laboratory streams from continuous exposure to

TABLE 2-1

Summary of Toxicological Properties of Diflubenzuron

1. Acute Oral Toxicity

Organism (Formulation)	Adverse Effects
Rat (Dimilin)	LD_{50} >4,640 mg/kg
Rat (Dimilin 25W)	LD_{50} >40,000 mg/kg
Rat (Dimilin, technical)	LD_{50} >4,640 mg/kg
Mouse (Dimilin)	LD_{50} >4,640 mg/kg
Mouse (Dimilin 25W)	LD_{50} >40,000 mg/kg
Mouse (Dimilin, technical)	LD_{50} >4,640 mg/kg

2. Acute Intraperitoneal Toxicity

Organism (Formulation)	Adverse Effects
Mouse (Dimilin, technical)	LD_{50} >2,150 mg/kg

3. Acute Dermal Toxicity

Organism (Formulation)	Adverse Effects
Rabbit (Dimilin 25W)	LD_{50} >4,000 mg/kg
Rabbit (Dimilin, technical)	LD_{50} >2,000 mg/kg

4. Acute Eye Irritation

Organism (Formulation)	Adverse Effects
Rabbit (Dimilin 25W)	Nonirritating.
Rabbit (Dimilin, technical)	Marginal irritant.

Table 2-1 (Continued)

5. Acute Inhalation Toxicity

Organism (Formulation)	Adverse Effects
Rat (Dimilin 25W)	LC_{50} >2.88 mg/l Category III
Rat (Dimilin, technical)	LC_{50} >35 mg/l

6. Subchronic Toxicity

Organism (Formulation)	Adverse Effects
Rat (Dimilin 99% purity)	Dose-related decrease in testosterone after 14 days some decrease at 28 and, 42 days, and no decrease at 96 days. Decrease in adrenal weight at 28 days after intragastric intubation with 15, 150, or 300 mg/kg.
Rat (diflubenzuron)	NOEL - 50 mg/kg; fed indirect 90 days.
Dog (diflubenzuron)	NOEL - 40 mg/kg; fed indirect 90 days.

7. Chronic Toxicity

Organism (Formulation)	Adverse Effects
Rat (diflubenzuron)	NOEL - 40 mg/kg; fed in diet 2 years.

8. Teratology, Reproduction and Fertility

Organism (Formulation)	Adverse Effects
Rat (diflubenzuron)	NOEL - 160 mg/kg. Fed in diet 3 generations. No adverse effect on growth reproduction, or litter size, weight, and mortality.

Table 2-1 (Continued)

8. Teratology, Reproduction and Fertility (Continued)

Organism (Formulation)	Adverse Effects
Rat (diflubenzuron)	No teratological effects. Exposed orally days 6 to 15 of gestation.
Rat (diflubenzuron)	No adverse effects on growth, reproduction, or litter size, weight, and mortality. Fed in diet 1 generation.
Rabbit (diflubenzuron)	No teratological effects. Exposed orally days 6 to 15 of gestation.
Sheep (diflubenzuron)	No effect on conception, lambing rate, birth weight RBC, WBC, hemoglobin, BUN, or bilirubin. Fed 0 to 250 ppm in diet to parents and offspring.

9. Carcinogencity

Organism (Formulation)	Adverse Effects
Rat (diflubenzuron)	Not carcinogenic.
Mouse (diflubenzuron)	NOEL - 50 mg/kg. No change in spontaneous tumor profile. Fed 4 to 50 mg/kg for 30 weeks.

10. Neurotoxicity and Behavior

Organism (Formulation)	Adverse Effects
No available data.	No available data.

Table 2-1 (Concluded)

11. Mutagenicity

Organism	Exposure	Adverse Effects
a. Prokaryotic Cells/Point Mutations Assays		
Salmonella typhimurium 4 strains	Diflubenzuron (technical)	Not mutagenic.
S. typhimurium 5 strains	Dimilin (25% wettable powder)	Not mutagenic.
S. typhimurium 5 strains	Diflubenzuron (recrystallized) and six proposed metabolites.	Not mutagenic.
b. Mammalian Cell Assays		
Mouse lymphoma L5178Y cells; point mutation assay)	Diflubenzuron (technical)	Not mutagenic.

Organism (Formulation)	Exposure	Adverse Effects
c. Mammalian in vivo Assays		
Mouse (diflubenzuron, technical)	Given 15, 150, or 1,500 mg/kg by gavage micronucleus test.	No increase in micronuclei or the ratio of mature/polychromatic erythrocytes in bone marrow over control animals.
Hamster (diflubenzuron) (10 day pregnant)	Given 1, 20, or 50 mg/100 g body weight. Host-mediated transplacental assay.	Not mutagenic. No increase in the number of transformed colonies or efficiency of colony formation in soft agar fetal cells.
Mouse (Dimilin)	Given 100, 500, 2,500 or 5,000 mg/kg.	Mutagenic. Increased number of chromosome aberrations as detected by metaphase analysis.

Table 2-2

Effects of Diflubenzuron on Target Organisms

Organism	Formulation	Effect	Reference
Western spruce budworm (Choristoneura occidentalis)	Difluron 25% WP	840 g/ha nearly complete kill at 2 weeks and 87% kill at 6 weeks	Gillette et al. 1978
Douglas-fir tussock moth (Orgyia pseudotsugata)	Difluron 25% WP	33.6 g/ha 100% mortality at 2 weeks and 51% at 6 weeks	Gillette et al. 1978
Mosquito (Aedes nigromaculis) (Culex tarsalis)	TH 6040	$LC_{50} = 7.2 \times 10^{-4}$ ppm; $LC_{90} = 3 \times 10^{-3}$ ppm; $LC_{50} = 7 \times 10^{-4}$ ppm; $LC_{90} = 1.4 \times 10^{-3}$ ppm	Miura and Takahashi 1974
Two-spotted spider mite (Tetranychus urticae)	Diflubenzuron	Metabolized slowly following absorption over 96 hours.	Franklin and Knowles 1981
Mosquito (Culex pipiens)	Diflubenzuron	Growth of larvae inhibited at 4 ppb; at 9 ppb large decrease in chitin synthesis.	Hajjar 1979
Spodoptera littoralis	Dimilin 25% WP 95% technical	Sterilization of 82% of females; reduced egg hatch; resistence developed after selective breeding.	Abbassy et al. 1980
Stable fly (Stomoxys calcitrans)	Diflubenzuron	Egg hatchability reduced 97% for 15 days.	Spates and Wright 1980

Table 2-2 (Continued)

Organism	Formulation	Effect	Reference
Gypsy Moth (*Lymantria dispar*)	Diflubenzuron	Abnormal molt, EC_{50} for 3rd instar = 0.052 ppm and at 5th instar = 0.009 ppm; EC_{50} for failure to pupate = 0.122 ppm; continued feeding resulted in lower EC_{50} = 0.006 ppm.	Abdelmonem and Mumma 1981
Plum curculio (*Conotrachelus nenuphar*)	Diflubenzuron 25% WP	No long lasting effect in field; in laboratory reduced pupation and eclosion at low dosages (0.5% a.i.).	Calkins et al. 1977
Gypsy Moth (*L. dispar*)	Dimilin	Effective 2 weeks after 2 applications.	Granett et al. 1976
Gnat (*Chaoborus astictopus*)	Diflubenzuron 25% WP	Number of lake larvae decreased by 99% within 3 weeks and remained low.	Apperson et al. 1978
Sheep blowfly (*Lucilia cuprina*)	Diflubenzuron	Inhibits chiton deposition in the cuticle; endocuticle layer appears abnormal.	Turnbull et al. 1980
Locust (*Locusta migratoria*)	Dimilin 25% WP	Dimilin increases fibrous appearance and weakening on the peritrophic membrane and reduction in weight.	Clarke et al. 1976
Mosquitos (Culicidae)	Diflubenzuron	22 to 56 g a.i./ha controls larval stages	Crosscurt 1978

Table 2-2 (Concluded)

Organism	Formulation	Effect	Reference
Midges (Chironamidae)	Diflubenzuron	110 g a.i./ha controls larval stages	
Flies (Muscidae)	Diflubenzuron	0.5 g a.i./m^2 controls feedlots or oral intake, 1 mg a.i./kg of food controls flies	
Horn fly (Haematobia)	Diflubenzuron	Ovicidal effects on females.	
Fungus gnats (Mycetophiladae)	Diflubenzuron	30 mg a.i./l controls mycelium or sporophores	
Humpback fly (Phoridae)	Diflubenzuron	30 mg a.i./l controls mycelium or sporophores	
Black fly (Simuliidae)	Diflubenzuron	Potential larvicide, simultaneous ovicidal activity.	
Lepidoptera	Diflubenzuron	7.5 to 200 g a.i./ha controls most species	
Cabbage looper (Trichoplusia)	Diflubenzuron	2.2 kg/ha controls	
Boll weevil (Anthonomus grandis)	Diflubenzuron	Males not affected, but egg hatch from females reduced 99%.	Mitchell et al. 1979

diflubenzuron. Stream pairs received concentrations of 0.1, 1.0, 10, or 50 mg/l of diflubenzuron. No effects were observed on the bacteria, oligochaetes, or gastropods at any of the test concentrations. Aquatic algae experienced increased biomass with the addition of 1 μg/l or more of diflubenzuron. Diatoms were the major algae group in all treatment levels, comprising more than 80 percent of the total. Effects on the algae were apparently indirect, resulting from the reduced invertebrate population rather than direct toxicity.

Diflubenzuron (≤100 ppm) and its metabolites (10 to 200 ppm) were added to cultures of Euglena gracilis Z. by Gattavecchia et al. (1981) to determine effects of the pesticide. Of diflubenzuron and its degradation products (4-chlorophenylurea, 2,6-difluorobenzoic acid, and 4-chloroaniline), only 4-chloroaniline was found to produce a considerable inhibition of Euglena growth.

Diflubenzuron showed some effect at higher levels only (higher than levels currently used for application); 4-chlorophenylurea and difluorobenzoic acid exhibited no inhibition in the growth of Euglena.

2.2 Invertebrates

The potential toxicity of diflubenzuron to invertebrate organisms have been studied in depth because of the potential widespread application of the pesticide within the the environment to control mosquitoes, midges and gnats, caterpillars, weevils, and gypsy moths, and its action in interfering with the deposition of chitin, one of the main components of the insect cuticle. Application sites are varied and include forests, agricultural areas, and commercial nurseries. In each of the application sites, target, as well as nontarget, invertebrate organisms may be exposed to the pesticide. Insects exposed to diflubenzuron die while trying to molt, because the new cuticle is not properly formed.

Appendix B describes toxicity data from studies on the exposure of invertebrates to diflubenzuron. Most recent data on target organisms are derived from field applications. Effects on nontarget invertebrates were observed mostly in tests conducted within in a laboratory or in small ponds or irrigation canals. Although useful in identifying potential problems or uncertainties requiring additional research, laboratory results do not necessarily reflect the potential effects of the pesticide in the natural environment. For example, in fresh water (i.e., in ecosystems and in field experiments where diflubenzuron was applied directly to the water) the effects on most taxa were less severe than effects resulting from acute toxicity tests conducted in the laboratory. This difference can be explained by environmental processes (e.g., dilution, adsorption, and microbial degradation of diflubenzuron) taking place at higher rates in field situations than under laboratory conditions. Additionally, in natural systems, interactions with other synthetic and naturally occurring chemicals, and changes in physical factors (e.g., pH, temperature, suspended particles, and water velocity) may affect assimilation and metabolism of the pesticide by invertebrates. These interactions could alter the tolerance organism to the pesticide. The effects of diflubenzuron overall are not nearly as severe as for standard insect control products (Rodrigues 1982).

Among target organisms, mosquitoes are extremely sensitive to diflubenzuron. In laboratory studies, very low LC_{95} values are usually recorded (in the order of 0.001 mg a.i. per liter [a.i./l]). This sensitivity may explain the findings of several field investigations that indicate that diflubenzuron substantially reduced mosquito populations in breeding areas. The diflubenzuron application caused reductions in populations of some less sensitive nontarget invertebrate organisms, but the nontarget species were not eliminated and could repopulate the area. The repopulation could result by the reproduction of the affected populations or by the colonization of individuals migrating from areas not treated (Dobrowski and Lambert 1985, Duphar 1985).

Recently, Martinat et al. (1988) reported on the effect of diflubenzuron on nontarget arthropod communities in a central Appalachian forest. Significant reductions were observed in macrolepidoptera and nonlepidopteran mandibulate herbivores. Sucking herbivorous insects, microlepidoptera, and predaceous arthropods were not affected. However, temporal trends and high between-tree variance in canopy arthropod communities would likely offset such reductions.

Aerial applications usually average 50 to 75 g a.i. per hectare (a.i./ha) to control insect pests in forests and tree plantations, but certain extremely susceptible insects (e.g., fall web worm, tent caterpillars, and gypsy moths) can be controlled with dosages as low as 25 grams per hectare (g/ha) (Duphar 1985).

Because of diflubenzuron toxicity to crab, shrimp, and other aquatic invertebrate organisms, the pesticide is classified as a restricted pesticide for use on forests and field crops. Cautionary statements are required on the diflubenzuron label warning of hazards to aquatic invertebrate organisms. The pesticide is allowed to be used for control of mosquitoes in temporarily flooded areas of pastures in central California, where the primary concern is the potential for the mosquitoes to carry diseases affecting humans acquired from breeding in wastewater from irrigation projects (U.S. EPA 1987).

Since diflubenzuron controls certain terrestrial insects by inhibition of chitin formation during larval development, the same affect has been suspected to occur with respect to aquatic larval insects. A recent study was conducted in a Louisiana coastal marsh to determine the affect of diflubenzuron on aquatic insects and other invertebrates (Farlow et al. 1978, Dobrowski and Lambert 1985). Six applications (28 g a.i./ha) of diflubenzuron over an 18-month period were observed to cause significant differences between treated and untreated populations. Statistically significant reductions were found in populations of water boatmen (Corixidae) and backswimmer (Notonectidae) nymphs, stalked winged damselfly (Coenagrionidae) naiads, adults of water scavenger beetles (Hydrophilidae), and amphipods. However, a recent study on the impacts of diflubenzuron in Newfoundland bog pools (Larson 1987) reported no statistically significant differences between control and spray pools for odonata, cladocerans, copepods, and aquatic coleoptera.

Some invertebrate organisms, including snails, predacious diving beetles, mayfly naiads, larval midges, and horse flies exhibited increases in population abundance upon exposure to diflubenzuron. It was suggested that the population increases could be attributed to the reduced abundance in populations of predator organisms. Similar reductions in populations of larval insects (without concurrent increases in populations of other organisms) have been reported by Miura and Takahashi (1974, 1975) and Hansen and Garton (1982a). Generally, the aquatic larvae affected shows a moderate decline for 1 to 2 weeks after treatment (Mohsen and Mulla 1982, McKague 1978).

The molting process and the formation of new integuments in crustacea has been observed to be inhibited by diflubenzuron (Dobrowski and Lambert 1985). The interference has occurred independent of life-cycle stage. Levels of only a few parts per billion (μg/l) of diflubenzuron or less affect the survival and reproduction of a number of different types of crustacea, including branchiopods, ostracods, copepods, mysidaceans, and decapods.

Concern with respect to the effects of diflubenzuron on crustacea focuses on the fact that diflubenzuron applied for mosquito control can travel into coastal waters where crustacea undergo frequent molts. Gulka, Doscher, and Watabe (1980) studied the effects of diflubenzuron on the barnacle (_Balanus eburneus_), which molts every few days as an adult. It was observed that diflubenzuron may lead to the excess production of the molting hormone, resulting in acceleration of the intermolt cycle. This acceleration, in some way, was associated with high mortality during the process of shedding the old exoskeleton, a diflubenzuron effect associated with target insects. Subsequent tests by Gulka and Gulka (1982) indicated that nontarget crustacea, both freshwater and marine, may be severely affected by diflubenzuron concentrations of only a few parts per billion or less. However, Wan and Wilson (1977) report an increase in some crustaceans (especially cladocerans) and no effect on copepods or ostracods coexisting with mosquito larvae in a freshnwater marsh.

Very little is known about possible concentrations of diflubenzuron to be expected in coastal waters and, thus, it is difficult to predict potential effects of diflubenzuron in coastal waters. One study attempted to characterize the diflubenzuron in coastal waters, but the test results were inconclusive with respect to reaching a general conclusion. The study, discussed above (Farlow et al. 1978), found that diflubenzuron treatment on a coastal marsh resulted in a reduction in population numbers of five taxa, an increase in population numbers of 15 taxa, and no statistically significant difference between treated and untreated populations of 27 other taxa. Weis and Ma (1987) found the larval stages of the horseshoe crab (Limulus polyphemus) were much more resistant to Dimilin than were crustacean larvae.

Based on studies conducted to date, in general, diflubenzuron has been determined to be relatively toxic to aquatic invertebrate organisms, as compared to other invertebrates (e.g., honey bees). The U.S. Environmental Protection Agency (1987) has determined that additional studies are required to complete a hazard assessment for aquatic invertebrates.

The effect of diflubenzuron on honey bees is of concern because of the high likelihood of exposure of the bees when diflubenzuron is used to control insect pests feeding on fruit and agricultural and horticultural crops. The acute contact toxicity to the adult bees is relatively low; i.e., the LD_{50} of diflubenzuron is greater than 30 μg/l and greater than 200 μg/l of Dimilin WP-25 per bee. In laboratory tests, Dimilin WP-25 fed directly to bee larvae in their cells, resulted in a LC_{50} of 3.7 mg a.i./l. When incorporated within the food via worker bees, 5.9 mg of diflubenzuron per liter of food did not cause any detrimental effect to the brood population (Duphar 1985).

In several experiments, hives with honey bees have been placed in areas treated with Dimilin used for the control of processionary caterpillars, gypsy moths, and sawflies. In one test, hives were caged within blossoming apple trees, which were subsequently sprayed. These various studies indicated that Dimilin does not affect the mortality of honey bees, pollen collection, activity of foraging bees, honey production, or brood development (Duphar 1985).

The activity of honey bees in cotton fields was not affected after repeated applications of Dimilin for the control of boll weevils (Dobrowski and Lambert 1985, in Johansen 1977). When applied for the control of pests in apple orchards in England, diflubenzuron application did not appear to harm honey bees. The diflubenzuron did not reduce the numbers of adult or larval bees, where as hexachlor and malathion killed many bees at lower concentrations. Even when diflubenzuron was sprayed on bees returning to hives, no adverse effects were observed. However, small amounts (0.11 to 0.30 μg/g) of diflubenzuron were found in samples of honey from hives sited in plots treated with the insecticide (Emmet and Archer 1980).

A Canadian study, Buckner et al. (1975), applied diflubenzuron by means of aerial application to a forest (350 g/ha). Honey bee populations were observed to remain normal following the application, whereas amphipods and beetle larvae in a stream within the treated area appeared to be impacted.

One case of honey bee mortality associated with diflubenzuron has been reported in the German literature. Egger (1977) observed that when diflubenzuron was applied aerially to forest trees, a mortality of 1.5 percent of larval honey bees was observed.

However, it was reported that professional bee keepers in the area of application did not report a negative effect on their bees (Dobrowski and Lambert 1985, in Egger 1977).

In another study, a 25 percent wettable powder formulation of diflubenzuron (100 mg/l) was supplied in water to colonies of honey bees. The relatively high concentration almost eliminated the brood. Treated bees consumed less water and pollen cake and produced less comb, brood, and new workers. However, treated bees had more eggs in their combs (Barker and Waller 1978).

In summary, honey bees appear not to be affected by normal field application rates of diflubenzuron (Peter 1987). Approved application rates are unlikely to harm either adult bees foraging on sprayed blossoms and/or flying through spray drift or immature bees developing within their colonies (Dobrowski and Lambert 1985, Emmett and Archer 1980).

Effects of diflubenzuron on soil-inhabiting mites are documented in Radwan and Abo-Korah (1978). Mites in suborders were exposed to a concentration of 250 g a.i. of diflubenzuron per acre of soil applied aerially. Each of these organisms experienced reductions in population averages of between 40 and 80 percent (see appendix B). In another study, a beneficial parasite, Chelonus blackburni, was exposed to diflubenzuron. In this study, Surulivelu (1982) reports 77 percent survivability after 24 hours of exposure, and 38 percent survivability for parasites released 72 hours after a 48-hour exposure. There are several reports that state the use of diflubenzuron does not severely affect beneficial species of soil mites (Ables, Jones and Bee 1977, Keever, Bradley and Ganyard 1977, Wilkinson et al. 1978).

Marshall (1979) also reported on effects of diflubenzuron on soil mites. Sixty-four species were exposed to field spray of diflubenzuron. Two weeks after application there was a reduction of six of these species. After a 3-month period, seven species were reduced. During this 3-month period, only three species had increased over the control. The reduction of individual species did not affect the total mite population over both the 3-month and a 1-year period; increases in other species seem to have compensated for reductions. Affected species and patterns of effects were not restricted to any particular trophic level.

2.3 Fish

Available documentation indicates that diflubenzuron is not toxic to fish. Appendix C summarizes studies that have been done to determine effects of diflubenzuron on fish. Clarke et al. (1977) proposed that chemicals with fish LC_{50}'s of less than one part per million (ppm) be considered "highly toxic;" between 10 and 100 ppm as "slightly toxic" and 100 ppm as "practically nontoxic." As evident in appendix C, current literature supports the finding that diflubenzuron is probably not toxic to fish.

Madder and Lockhart (1978) performed a study of the effects of chronic flow through test (96 hour) of Dimilin 25W on rainbow trout. Although LC_{50} values for diflubenzuron are significantly greater than concentrations normally applied for insect control (i.e., ≥135 mg/l LC_{50} vs. ≤0.05 mg/l) sublethal effects could occur. Serum analyses of fish exposed to diflubenzuron were inconclusive with regard to sublethal pathological effects.

Laboratory studies with bullheads and sunfish by Buckner et al. (1975) indicated no toxic effects of exposure up to 500 ppm of Dimilin 25W for 2 weeks. Hanson and Garton (1982) used single-species toxicity tests to assess the effect of diflubenzuron on steelhead trout, fathead minnow, and guppy. The maximum concentration used in the tests was 45 µg/l, and no effects to any of the fish were noted.

In a series of 96-hour immersion studies, at temperatures between 7 and 22 °C, Johnson and Finley (1980) reported LC_{50} values of more than 100 ppm for rainbow trout, and more than 50 for brook trout, channel catfish, and blue gill, in 95 percent (technical) diflubenzuron. Exposure to the 25 percent wettable powder formulation of diflubenzuron yielded LC_{50} values of 57 and 240 ppm for cutthroat trout and rainbow trout respectively; and more than 100 ppm for fathead minnows, channel catfish, and bluegill. The most sensitive species was yellow perch, which has a 96-hour LC_{50} of 25 mg/l in 95 percent diflubenzuron. According to EPA classifications, LC_{50} values of between 10 and 100 are "slightly toxic" for freshwater fish and values greater than 100 are "practically nontoxic."

Alterations in test conditions such as pH, temperature, and hardness did not affect the toxicity of diflubenzuron. No adverse effects were observed when eyed eggs and fingerlings of rainbow trout were exposed in a flow-through system for 30 days to concentrations of technical grade diflubenzuron of 0.029 to 0.30 mg/l.

2.4 Birds

Although limited, available data on the effect of diflubenzuron on birds, indicate low or no level of toxicity to avian populations.

In an experimental aerial application of 350 g/ha, effects of diflubenzuron on small songbirds were studied and reported in Buckner et al. (1975). Populations were assessed daily on sprayed and control plots for 5 days before and 5 days after application of diflubenzuron. Daily population fluctuations were relatively small. The study concluded that diflubenzuron applications had no observable effect on the population of the songbirds.

Another study involving insectivorous songbirds exposed to aerial application of diflubenzuron is reported in De Reede (1982). Three species (great tits, blue tits, and tree sparrows) were examined to determine potential effects of diflubenzuron, as a result of ingestion of contaminated caterpillars. Historical data were used to estimate residues of diflubenzuron in caterpillars and maximum daily intake by birds. A control area was also used to monitor feeding, nesting, and foraging behavior. Results indicate no observable effect on breeding success and growth of nestlings of great tit and tree sparrows in orchards sprayed with diflubenzuron. The maximum possible daily intake of diflubenzuron by wild nestings was estimated at 15 mg/kg body weight/day for the great tit, 10 mg/kg body weight for the red-backed shrike. The author concludes that poisoning of birds in orchard and shade trees by diflubenzuron, properly applied for pest control (i.e., 0.06 lb a.i./acre), is highly improbable.

Several studies (summarized in appendix D) were performed to determine effects of diflubenzuron on chicken populations. The study reported in Smalley (1976) involved feeding baby chicks for 13 weeks with diflubenzuron (TH 6040). Results indicated an increase of fat deposition in female chicks. The results showed body weights of the diflubenzuron-fed female chicks to be in excess of a 100 percent increase over that of nondiflubenzuron-fed female chicks and test males. The test males did not appear to mature properly. Their inability to develop combs, wattles, feathers, and voice was related to a dose-related decrease in testosterone.

Cecil et al. (1981) reported on effects on production and reproduction in diflubenzuron-fed White Leghorn hens. Mature hens were fed 10, 50, 100, and 500 ppm diflubenzuron for 8 weeks. No adverse effects were reported on feed consumption, body weight, egg production, or progeny performance. Diflubenzuron accumulated in eggs to levels approximately 0.04 to 0.004 of the dietary level with a lesser accumulation in the liver and muscle. After 5 weeks off the treatment, diflubenzuron was not detectable in the egg, liver, fat, or muscle tissues of hens fed any level of the compound. Miller et al. (1975) results were confirmed with the above-mentioned discussion on White Leghorn hens.

Kubena (1982) reports on the effects of diflubenzuron on reproduction in chickens. Diflubenzuron was fed to male and female chickens from 1 day of age through a laying cycle (approximately 30 days), at levels of 0, 2.5, 25 and 250 ppm. No effects were observed on egg production, egg weight, eggshell weight, fertility, hatchability, and progeny at any of the diflubenzuron levels fed. These findings corroborated the earlier study of Miller (1976) in which fertility and hatchability were not affected in diflubenzuron-fed (10 ppm) chickens. Miller (1976) also reported some accumulation of diflubenzuron in eggs as well as liver.

Opdycke et al. (1982) reported on *in vivo* and liver microsomal metabolism of diflubenzuron by White Leghorn, Rhode Island Red, and Barred Plymouth Rock chickens. The chickens were fed 5 mg/kg (0.04 ppm) technical (95 percent) diflubenzuron for 312 days (this dose represented 10 times the normal dose expected). Results confirmed previous studies in which White Leghorns exhibited a 2-fold higher egg residue than the other breeds. Results of the study also indicated that diflubenzuron is not readily metabolized in these chicken breeds and that most unaltered diflubenzuron (80 percent) was excreted rapidly (within 12 days). Laboratory tests with liver microsomal preparations showed less than 10 percent conversion to metabolites.

2.5 Mammals

The potential health hazards from acute and chronic exposure to diflubenzuron has been evaluated in laboratory animals such as rats, mice, rabbits, dogs, and sheep. The results of the toxicity tests summarized in table 2-1, appendix E, and appendix G are discussed below.

Diflubenzuron is considered to have a low acute toxicity on the basis of animal studies. There is no concern for carcinogenic or teratogenic hazards with diflubenzuron. On the basis of five test systems, diflubenzuron is not mutagenic.

2.5.1 Acute Toxicity

Acute toxicity data for diflubenzuron are presented in table 2-1 and appendix E. By oral, dermal, and inhalation routes, diflubenzuron formulations have low toxicity. Technical Dimilin is marginally irritating while Dimilin 25W is not.

2.5.1.1 Oral. An unspecified Dimilin formulation, Dimilin 25W, a 25 percent wettable powder, and technical Dimilin were tested for acute oral toxicity to rats and mice. The LD_{50}'s were identical in these species and were >4,640 mg/kg for Dimilin (unspecified), >40,000 mg/kg for Dimilin 25W, and >4,640 mg/kg for technical Dimilin (Dobrowski and Lambert 1985, Ag Chemicals 1983). The U.S. Environmental Protection Agency classifies the acute oral toxicity of Dimilin in Category IV (U.S. EPA 1987). No information on the nature of exposure was available for these oral studies.

2.5.1.2 Intraperitoneal. Technical Dimilin administered to mice by the intraperitoneal route yielded an LD_{50} >2,150 mg/kg (Dobrowski and Lambert 1985, Wegler 1980). No other experimental details were reported.

2.5.1.3 Dermal. Three dermal toxicity studies in rats are identified. In one study, Dimilin 25W was not irritating and the LD_{50} was greater than 18,650 mg/kg (Ag Chemicals 1983, Wegler 1980). In the second study on Dimilin 25W, the LD_{50} was greater than 4,000 mg/kg and Dimilin 25W was classified in toxicity Category III (U.S. EPA 1987). In the third study, technical Dimilin yielded a dermal rat LD_{50} greater than 2,000 mg/kg (Ag Chemicals 1983, Wegler 1980). No other experimental details were reported.

2.5.1.4 Eye Irritation. Two eye irritation studies were conducted in rabbits. In the first study, Dimilin 25W caused no irritation and in the second study, technical Dimilin caused marginal irritation (Ag Chemicals 1983). No other experimental details were reported.

2.5.1.5 Inhalation. Two inhalation studies were conducted in rats. In the first, Dimilin 25W was mildly toxic, yielded an LC_{50} greater than 2.88 mg/l, and was classified in toxicity Category III (U.S. EPA 1987). In the second study, technical Dimilin was less toxic yielding an LC_{50} greater than 35 mg/l (Ag Chemicals 1983).

2.5.2 Subchronic Toxicity

Two subchronic toxicity studies were reported in rats and one in dogs. Decreases in plasma testosterone and adrenal weights and increases in methemoglobin and sulfhemoglobin were the only findings. Subchronic oral toxicity testing of diflubenzuron was reported by Patel and Santolucito (1980) in male rats. Exposure to diflubenzuron was associated with decreased plasma testosterone levels and adrenal weights, but no additional toxicity was reported. Following daily exposure to Dimilin (99 percent pure) to 25-day-old male Long-Evans rats by intragastic intubation at 0, 15, 150 or 300 mg/kg in a corn oil vehicle, 8 animals from each treatment group and 15 animals from the control group were sacrificed at 14, 28, 42, or 96 days for histological and pathological examination. Plasma testosterone levels dose-related decreases showed after 14 days of treatment, but returned toward control levels after 28 and 42 days of treatment, there were no significant differences from control levels at any other treatment time. No significant decreases in testicular, prostatic, or body weights were observed. Histological and pathological examinations of treated animals showed no abnormalities that could be related to diflubenzuron treatment. Patel and Santolucito (1980) concluded that the decreased adrenal weights in one treatment group were of minor importance, and that since the decreased plasma testosterone levels returned to normal levels after 96 days of treatment and no histological changes in interstitial or germinal cells were observed, the transient depression of plasma testosterone levels exposure to produced by diflubenzuron (Dimilin) resulted in no adverse effects on the development of the reproductive organs of male rats.

In a second subchronic study in rats, dietary supplements of 3.125 to 200 mg of diflubenzuron were provided for 90 days (Wegler 1980). The NOEL was reported to be 50 mg/kg, but the parameters measured were unspecified.

The third subchronic toxicity study was in beagle dogs. The dogs were fed diets containing 20 to 160 mg/kg of diflubenzuron for 90 days (Wegler 1980). The NOEL was reported to be 40 mg/kg. Marginal increases in methemoglobin and sulfhemoglobin were observed at 160 mg/kg. No other experimental details were reported.

2.5.3 Chronic Toxicity

One chronic toxicity study was reported in which both male and female rats were fed diets containing 10 to 160 mg/kg of diflubenzuron for 2 years (Wegler 1980). The NOEL was 40 mg/kg. A marginal increase in methemoglobin was observed in both sexes at 160 mg/kg. No other experimental details were reported.

2.5.4 Reproductive and Teratogenic Effects

Five studies carried out in rats, rabbits, and sheep showed no adverse reproductive or teratogenic effects of diflubenzuron. In a three-generation study in rats fed diets containing 10 to 160 mg/kg, the NOEL was 160 mg/kg and there were no adverse effects on growth, reproduction, mating performance, pregnancy rate, duration of gestation, total litter loss, litter size, litter and mean pup weight, pup mortality, and incidence of abnormalities (Wegler 1980, Dobrowski and Lambert 1985). In a second study, diflubenzuron (up to 100,000 ppm) fed rats for one generation (Dobrowski and Lambert 1985) produced no adverse effects on growth and reproduction, and all of the effects in the previous study were observed. In the third study, rats were exposed orally to 1 to 4 mg/kg of diflubenzuron on days 6 to 15 of gestation (Dobrowski and Lambert 1985). No teratological effects were observed. No other experimental details were provided.

In one rabbit study, the animals were exposed orally to 1 to 4 mg/kg on days 6 to 15 of gestation and no teratological effects were observed. (Dobrowski and Lambert 1985). No other experimental details were reported. In the fifth study, sheep were fed 100 ppm diflubenzuron in the diet for 7 to 8 months beginning 1 month before breeding and ending when lambs were weaned (Escobar et al. 1980). The lambs were fed 0 to 250 ppm in the diet. There were no adverse effects on conception, lambing rate, birth weight, and live lambs per ewe. Lambs fed 0 to 250 ppm had no adverse effects with respect to weight gain, food intake, food conversion or clinical parameters measured in blood including WBC, RBC, BUN, hemoglobin, protein, packed cell volume, and bilirubin.

2.5.5 Carcinogenicity

Two studies reported no carcinogenic effects of diflubenzuron in rats and mice. Male and female rats administered diflubenzuron up to 10,000 ppm showed no carcinogenic response (U.S. EPA 1987). Male and female rats fed diets containing 4 to 50 mg/kg of diflubenzuron for 80 weeks had no change in their spontaneous tumor profiles and the NOEL was 50 mg/kg (Wegler 1980). No other experimental details were reported in these studies.

2.5.6 Neurotoxicity

No data were reported on neurotoxicity or behavior.

2.5.7 Mutagenicity

The genetic effects of diflubenzuron, its formulations, and six prop osed metabolites are presented in table 2-1 and appendix G. The data indicate that neither diflubenzuron nor its metabolites are mutagenic to bacteria or mammals.

Bacterial (prokaryotic cells) mutagenicity assays are designed to detect point (i.e., single gene) mutational events. MacGregor et al. (1979) reported that technical grade diflubenzuron induced no increase over the spontaneous reversion frequency in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 in the presence or absence of metabolic activation enzymes. A commercial preparation of diflubenzuron, Dimilin 25W (25 percent wettable powder), tested for mutagenicity by MacGregor et al. (1979) in these *S. typhimurium* strains, also produced negative results. Seuferer et al. (1979) found that recrystallized diflubenzuron produced no increase above the spontaneous reversion frequency of *S. typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 at a dose of 1,000 µg/plate in the presence of metabolic activation. In addition, they tested six proposed metabolites of diflubenzuron for mutagenicity. These proposed metabolites were 4-chloroaniline, 4-chlorophenylurea, 4-chlorophenol, acetanilide, 4-chloroacetanilide, and 2,6-difluorobenzoic acid. All of these proposed metabolites were negative in all five tester strains of bacteria. Diflubenzuron has also been tested for mutagencity in mammalian cells in vitro. MacGregor et al. (1979) tested technical grade diflubenzuron in the L5178Y lymphoma cell assay in the presence and absence of metabolic activation. This assay tests for the loss of function of the enzyme thymidine kinase. At concentrations up to 300 µg/ml, diflubenzuron did not increase the mutation frequency of the L5178Y mouse lymphoma cells. The positive control, dimethylnitrosamine, increased the mutation frequency in this assay, confirming the activity of the metabolic activation system. The concentrations of diflubenzuron used in this study did not reduce the plating efficiency of the L5178Y cells, indicating that these results are not due to any possible cytotoxic effects of diflubenzuron.

Mutagencity studies of diflubenzuron in in vivo assays have utilized mice and hamsters. MacGregor et al. (1979) administered technical grade diflubenzuron to groups of 5 male Swiss-Webster mice at 15, 150, and 1,500 mg/kg by gavage. Bone marrow smears were examined for micronuclei and the ratios of mature to polychromatic erythrocytes, both of which are markers for chromosome loss or breakage resulting from mitotic abnormalities in developing erythrocytes. No alterations in these markers were found in any treatment group as compared to control animals. These data indicate that diflubenzuron does not induce chromosome damage in mice at doses up to 1,500 mg/kg. Triethylenemelamine, a potent inducer of micronuclei, was used as a positive control in this study and did produce a significant increase in micronuclei formation in these experiments, demonstrating that these mice were sensitive to chemicals that induce the formation of micronuclei. However, it should be pointed out that this assay is not sensitive to increases in the incidence of micronucleated erythrocytes of less than three to four times background, and the formation of micronuclei below this frequency may therefore go undetected.

Quarles et al. (1980) tested diflubenzuron for mutagencity in vivo in the host-mediated transplacental assay. This assay tests for the frequency of morphological transformation and efficiency of growth in soft agar of fetal cells following in utero exposure to mutagens. In this assay, fetal tissue of pregnant animals is exposed in utero to a chemical administered to the mother. The fetal tissue is then digested into single cells and cultured in vitro. The surviving cells are analyzed for their frequency of morphological transformation and efficiency of growth in soft agar, two characteristics thought to be associated with preneoplastic cells. Diflubenzuron treatment of 10-day pregnant hamsters at up to 50 mg/100 g body weight intraperitoneally did not increase the frequency of transformed colony formation or the efficiency of colony formation in soft agar of fetal cells as compared to fetal cells from untreated hamsters. Three positive control mutagens did produce increased frequency of transformed colony formation and growth in soft agar following treatment _in vivo_. These data support the other studies that demonstrate diflubenzuron is not mutagenic.

The only study that reports positive mutagenicity data on diflubenzuron is from the Soviet Union; Kurinnyi et al. (1982), dosed mice with Dimilin at 100, 500, and 2,500 mg/kg, by an unspecified route of exposure, and analyzed the number of chromosome aberrations in bone marrow cells. They found an increased number of chromosome aberrations in the bone marrow of animals treated with diflubenzuron, but there was not a clear does-response relationship between the number of chromosome aberrations and the dose of diflubenzuron administered. Therefore, the data in the report by Kurinnyi et al. (1982) are not adequate to support a conclusion that diflubenzuron is a chromosome mutagen.

3.0 Environmental Fate

Diflubenzuron, an insect growth regulator (IGR) used for insect pest control, functions by inhibiting chitin synthesis during molting. The compound is readily degradable in the environment and does not persist in soil or water (Pieper et al. 1977, Blumberg 1986, Van Den Berg 1986). Willcox and Coffey (1978) reported that only 1 of 48 samples showed a detectable residue. Diflubenzuron exhibits virtually no mobility either in the soil or from treated soil to plants. There is some persistence on plant surfaces; these residues decline through dilution by growth of the plant. It does not photolytically degrade on nonbiological surfaces.

Diflubenzuron has a short half-life of 0.5 to 1 week, which is apparently unrelated to soil type but dependent on the microbial activity of the soil and particle size of the diflubenzuron formulation (Verloop and Ferrel 1977; Seuferer et al. 1979). The half-life of diflubenzuron in natural waters (pH 6 to 9) is less than 1 week. However, under very acidic conditions (pH 4.0), degradation was not detected even after 56 days (Ivie et al. 1980). Bioaccumulation for aquatic organisms during exposure generally does not exceed 100 times over ambient levels and is reversible after removal of exposure.

Studies on the fate of diflubenzuron in air have not been reported (Dobrowski et al. 1985).

3.1 Bioaccumulation and Metabolism

Diflubenzuron is highly biodegradable in biological systems and most animal species and does not bioaccumulate. Degradation products include 2,6-difluorobenzoic acid (DFBA), 4-chlorophenylurea (CPU), and 4-chloroaniline (PCA), a minor degradation product.

3.1.1 Plants

One reason that diflubenzuron is so effective in pest control ("worms" and insect larva) is the stability of the compound on the foliage being treated. A spray residue does not enter plant cells and is not easily washed off by rain (Peter 1987). Residues on vegetation can be reduced by application of granular formulation of diflubenzuron. Studies have been performed to determine the environmental fate of diflubenzuron and its metabolites on vegetation, using different formulations.

Schaefer and Dupras (1979) report on the persistence of diflubenzuron on pasture grasses after being applied as an aqueous spray of 0.05 lb a.i./acre (3.8 ppm). Samples were collected before application, 1 hour after application, and on days 3, 7, 14, 22, 28, 35, and 42 after application. Results of the study showed no decrease in the residue after 3 days and a decrease to an estimated 50 percent of the initial concentration after approximately 12 days. After 42 days, 8 percent of the initial concentration was detected in the sample.

The pasture grass investigation corroborated previous results on persistence of diflubenzuron on vegetation. Schaefer and Dupras (1979) reported on several studies in which diflubenzuron was applied in a variety of formulations (i.e., wettable powder, 1 percent Attaclay granules, sand granules) to determine residues in pasture soil, vegetation and water, following aerial applications. Results indicated a reduction in persistence on vegetation when using a granular application. The experiment also demonstrated effectiveness of the diflubenzuron against pests (mosquitos) without the production of undesirable residues on vegetation.

Studies on the fate of diflubenzuron on cotton are reported in Bull (1980), Mansager et al. (1979) and Bull and Ivie (1978). These studies indicate that, regardless of application method, very little of diflubenzuron is absorbed by cotton leaves. Analyses associated with these studies showed that 98 percent of diflubenzuron recovered from leaf samples was unchanged. These results indicated that diflubenzuron is highly resistant to decomposition either by photodegradation on leaf surfaces or by metabolism within the cotton leaves. Similar results have been reported in studies with soybeans, cabbage, corn, and apples (Verloop and Ferrell 1977).

Bull and Ivie (1978) report on effects of 1,000 ppm a.i. ^{14}C-labeled Dimilin 25W applied directly to field-grown cotton leaves. After 14 days, almost 90 percent of the applied dose was unabsorbed; only about 4.8 percent was recovered from internal extracts and <1 percent could not be extracted from leaf tissues. After 21 days and a heavy rainfall, 23 percent of the applied diflubenzuron was still on treated leaf surfaces. In the greenhouse portion of the study, (protected against rain) more than half the applied 500 ppm Dimilin 25W was still unabsorbed after 28 days exposure of the leaves to summer sunlight; only about 7 percent was associated with the leaf residue; and the remainder (38 percent) was presumably lost through volatilization or other "weathering" factors). Bull and Ivie (1978) also performed tests on cotton plants sprayed with multiple applications of ^{14}C-labeled diflubenzuron (25 percent wettable powder, diluted) equivalent to 70 g of a.i. in 9.35 l oil and 93.5 l water/ha. Two plots were treated at 5-day intervals; one with 6 applications, the other with 10. Results of combustion analyses indicated little accumulation of radiocarbon in the seeds collected from both plots. Less than 0.01 ppm were found in seeds of the first plot and about 0.02 ppm in those from the 10-day plot. Radioactive residues were relatively high in some parts of the plants, especially leaves that were on the plant during treatment (107 ppm after 10 treatments, 38 after 6 treatments). New leaves had residues of 0.60 to 1.38 ppm and the stalk, root, and calyx had residues of 0.76 to 3.29 ppm; the higher levels were found in the plots treated 10 times.

Mansager et al. (1979) report on leaf and soil studies of diflubenzuron on cotton plants. Three experiments were done to study the fate of ^{14}C-labeled diflubenzuron on cotton leaf surfaces. One study involved three applications of 600 g (aqueous spray) diflubenzuron/plant/application, 7 days apart, with 3 plants harvested at 7, 14, and 28 days and 3 plants harvested 48 days after the last treatment. The other two experiments involved only a single application: one aqueous suspension, one oil emulsion. All plants in these latter two experiments were harvested at 7, 14, and 28 days after the last treatment. Results showed that absorption, metabolism, and translocation of ^{14}C-diflubenzuron were minimal. Only trace concentrations were found in the leaves, stems, bolls, or squares (<.01 to .03 ppm) of the new cotton tissues 48 days after treatment.

Schroeder et al. (1980) reported on fate of diflubenzuron on a citrus grove. A 15-year-old grove was sprayed aerially with diflubenzuron plus oil at 350 g/a.i./ha for a total of eight applications over a period of 7 months. Results of sampling showed diflubenzuron residue in unwashed fruit, washed fruit, dried pulp, peel fruit, chopped peel, and oil (0.34, 0.11, 0.26, 0.31, 0.12, and 20.55 ppm, respectively). No detectable residue (<0.05 ppm) of diflubenzuron was found in the finisher pulp, fruit juice, pressed liquor, molasses, prewash or afterwash water, and emulsion water fractions. No residues of diflubenzuron metabolites, 4-chlorophenylurea, or 4-chloroaniline (<0.05 ppm), were found in the citrus fractions, nor in the prewash or afterwash water. The results from the extracted peel and oil indicated that the majority of the diflubenzuron found in the peel was actually in the oil.

In another study of diflubenzuron effects on plants, Austin and Hall (1981) reported on apple foliage and fruit exposed over a period of 3 years to concentrations of 50, 100, 250, and 208 mg a.i./l (25 percent wettable powder). Leaf and fruit samples extracted and analyzed using a direct-insertion probe technique revealed minimum quantifiable residues of 1.0 and 0.025, respectively. Diflubenzuron residues on leaves ranged from 0.14 to 1.34 mg/cm^2 in 1977 and 0.18 to 0.83 mg/cm^2 in 1979. Residues on fruit in 1977 for all application rates ranged from not detected (for the last month sampled) to 0.45 μg/cm^2 (for the highest concentration applied). None of the conversion products of diflubenzuron (4-chloroaniline, 4-chloroformalilide, 4-chlorophenylisocyanate, 4-chlorophenylurea, 4-chloconitrobenzene, cis- and trans-4-dichloroazobenzene, 4,4-dichloroazoxybenzene, and 2,6-difluorobenzoic acid) were identified in leaf or fruit extracts.

Persistence of diflubenzuron in stored wheat was studied and reported in Mian and Mulla (1982). Technical diflubenzuron (95 percent) was applied using aqueous spray at concentrations of 1, 5, and 10 ppm to wheat grain. Grain samples were analyzed at 0 and 1 week and 1, 5, 10, and 23 months post-treatment. Results indicate that residues of 0.88 ppm in stored wheat at 1 week decreased to 0.59 ppm at 23 months for 1 ppm diflubenzuron applied; residues of 4.07 ppm at 1 week decreasing to 2.75 ppm at 23 months for 5 ppm diflubenzuron applied; and residues of 8.76 ppm at 1 week decreasing to 5.00 ppm at 23 months for 10 ppm diflubenzuron applied.

3.1.2 Animals

Studies of diflubenzuron in several animal species indicate that absorption from the gastrointestinal tract is minimal. Absorbed material is extensively metabolized. The majority of the ingested compound is nonabsorbed material excreted unchanged in the feces. There is no significant accumulation of diflubenzuron in the body. Studies indicate that the fraction of an oral dose of diflubenzuron absorbed by the intestines diminishes considerably with increasing dosage.

Appendix H describes the results of studies on the accumulation and metabolism of diflubenzuron in animals. These studies include studies on fate of diflubenzuron in rats, swine, cows, and sheep and studies in target and nontarget invertebrates.

Stoolmiller (1978) cultured rat C-6 astrocytoma cells in acetone-based medium containing up to 31 ppm diflubenzuron. No changes were observed in cell morphology and adhesiveness, rates of cell division, or vital staining as a result of addition of diflubenzuron. In addition, comparative biosynthesis studies showed no inhibition of production or function of glycosaminoglycans of rat cells in the diflubenzuron treated medium.

Willems et al. (1980) fed radioactively labeled diflubenzuron to Wistar rats about 200 g body weight. In rats fed 5 mg/kg diflubenzuron, after 72 hours, only a small fraction of the dose remained in the carcass. In rats with cannulated bile ducts and in normal rats, about 20 percent of the radioactive labels were excreted in the urine; an average of one-third of the dose was recovered in the bile of these rates. The sum of the excretions (urinary and biliary) indicated absorption of about half the administered dose. To determine intestinal absorption as a function of dose level, normal and bile-cannulated rats were fed radiolabeled diflubenzuron. In normal male (M) and female (F) rats, the experiment lasted 120 hours and the cumulative excretions (urine plus feces) were 88.3, 86.7, 92.4, 91.1, 91.2 and 84.5 percent of administered doses for 4(F), 16(F), 48(M), 128(F), 128(M) and 1,000(M) mg/kg respectively. In the female rats with cannulated bile ducts, 72 hours after the experiment, cumulative excretion of diflubenzuron in urine plus bile, as percent of administered dose was 78.1, 78.1, 84.0 and 78.8 for 4, 16, 125, and 900 mg/kg. Identification of major urinary metabolites of diflubenzuron included 2,6-difluorobenzoic acid and two others. Neither 4-chlorophenylurea as such or 4-chloroaniline were detected. Indications from patterns of the radiolabeled C in urine, were that 4-chlorophenylurea was further metabolized.

Opdycke et al. (1982) fed 5 mg/kg radiolabeled diflubenzuron at 12-hour intervals to a female Duroc-Poland China pig as an oral dose. Radioactivity analysis of urine and feces collected at 12-hour intervals were performed for 11 days. Results of these analyses revealed over 82 percent of the dose in feces and 5 percent in urine. Samples of organ, muscle, fat, and bone tissues and blood were taken at 11 days. The highest levels of diflubenzuron residue were found in the gall bladder (0.43 ppm) and in fat tissues of the omentum (0.30 ppm), a fold of the peritoneum. All other tissue residues were found to be less than 0.20 ppm. Swine urine metabolites of diflubenzuron identified included 4-chloroaniline (1.03 percent dose), 2,6-difluorobenzamide (0.83 percent), 4-chlorophenylurea (0.82 percent), and 2,6-difluorobenzoic acid (0.2 percent).

Three dairy cows were fed TH 6040 at different concentrations: one cow at 1 mg/kg for almost 4 months; the second cow at 1 to 8 mg/kg for 2-week periods, then 16 mg for 3 months; and the third cow at 0.25 mg/kg for 4 months. No adverse effects on the cows were observed during the feeding period. No residues (<0.02 ppm) were detected in milk after 1 to 8 mg/kg for a 2-week period; at 16 mg, a trace of diflubenzuron was detected in a small sample (200 g milk) and 0.02 ppm was detected in a larger sample (800 g). The manure content of diflubenzuron of the cow fed 0.25 mg/kg decreased over time at a hyperbolic rate; the half-life was 4 to 5 days. The diflubenzuron in the feces of the cow fed 16 mg/kg decreased somewhat over time but more than half the original amount was still present at 45 days. Residues of diflubenzuron in fat and tissue samples were highest in omental fat (0.1 ppm) but detected in all four fat samples and liver.

Ivie (1978) reported on fate of radioactively labeled diflubenzuron administered orally and dermally to cattle and sheep. A lactating cow was fed, via a stomach tube, 10 mg diflubenzuron/kg of body weight. Total urine and feces samples were then collected at 24-hour intervals, and the cow was machine-milked every 12 hours. During the 7-day post-treatment period, about 85 percent and 15 percent of the administered dose was excreted in the feces and urine, respectively. Only 0.2 percent of the diflubenzuron given the cow was secreted into the milk. Maximum milk residues of 0.8 ppm occurred in the samples collected at 24-hours after dosing; after 3 days the compound was undetectable (<0.1 ppm). Only the liver contained appreciable residues (2.3 to 3.6 ppm). Treatment of sheep was similar to that of the cow. However, 4 sheep were treated: 2 normal, 2 cannulated; 1 of each were fed orally at 500 mg/kg, the other two were tube-fed as with the cow. Results of feces and urine samples of the 10 mg/kg-fed sheep indicated a larger proportion diflubenzuron (19 to 40 percent) in the urine then in the cow. The bile was the major route of elimination in the cannulated sheep. Sheep treated with the 500 mg/kg dose eliminated a much smaller proportion of the labeled compound in urine and bile. Results of tissue analysis (4 days after treatment) in the sheep (10 mg) were similar to those of the cows above. Tissues from the sheep fed 500 mg were not analyzed. Considerable quantities of metabolites and unextractable radiocarbon were also detected in feces of all treated animals except sheep with cannulated bile duets. In their case, the excreted radiocarbon was almost totally extracted and consisted only of unmetabolized diflubenzuron.

In a related study, Ivie (no date) reported that unlike the effective metabolism demonstrated in cattle and sheep, the stable fly and housefly are much less effective at metabolizing diflubenzuron. Adult stable flies (*Stomoxys calcitrans* L.) and houseflies (*Musca domestical* L.) were treated topically on the ventral side of the abdomen with 2 μg in 1 μg acetone/fly of radiolabeled diflubenzuron. Four days after treatment, samples were taken and analyzed. Results indicated that 40 to 50 percent of the applied radiocarbon remained on the treated stable flies and 20 to 40 percent on the houseflies. In the stable flies, 35 to 40 percent of the dose was found to be excreted and about 50 percent from the houseflies. More than 97 percent of the radiocarbon recovered from the stable flies and their excreta was unmetabolized diflubenzuron, but houseflies degraded 10 percent of their dose. Metabolites corresponding to 2,6-difluorobenzamide, and 4-chlorophenylurea were detected in samples from both species, whereas trace quantities of 4-chloroacetanilide and 2,6-difluorobenzoic acid were seen in the excreta of only the stable flies.

In addition to reporting the results described above, Ivie and Wright (1978) examined fate of diflubenzuron in eggs from stable flies and houseflies. After 3 to 4 days post-treatment (2 μg/l), females of both species were stimulated to lay eggs. Samples of at least 100 eggs from each treatment group and replicate were studied for hatchability and survivability. Female stable flies secreted almost 1 percent of the dose into eggs and untreated females caged with treated males secreted 0.5 percent of the dose into eggs. Treated houseflies excreted 0.17 percent of the dose in eggs; untreated females caged with treated males excreted 0.1 percent of the diflubenzuron dose in eggs. Both species produced eggs at a rate of 84/female. Hatchability was severely inhibited in the stable flies treated with diflubenzuron. None that did hatch reached adult stage. Treated houseflies did not experience inhibition of hatchability, although subsequent development was severely inhibited (<14 percent hatched eggs reached adult stage). Untreated flies of one sex held with treated flies of the opposite sex contained appreciable residue 4 days after treatment. Untreated stable flies and houseflies exhibited 3 to 4 percent and 1 to 1.5 percent of the applied dose respectively. Total recovery ranged between 72 to 92 percent.

Chang (1978) reports on metabolism of radiolabeled diflubenzuron injected into each of 100 female houseflies. After 72-hours, 18.4 percent of the applied dose was recovered from the flies; 74 percent was unchanged diflubenzuron and 4.4 percent was diflubenzuron conjugate. In the excreta, 16.5 percent of the applied dose was recovered, all as diflubenzuron conjugates.

Metabolism of diflubenzuron in the boll weevil is reported in Chang and Stokes (1975). Boll weevils (ebony strain) were injected with 4 μg/weevil of radiolabeled diflubenzuron, (^{14}C). After 4 days of feeding, a total of 91.1 percent of the applied dose was recovered; 63.2 percent from the treated weevils and 27.9 percent from their excreta. The extractables were 57.7 percent from treated weevils and 24.7 percent from their excreta. In the weevil, 57.3 percent of the ^{14}C was recovered as unchanged diflubenzuron and 0.4 percent was recovered as conjugates. In the excreta, about 2 percent each of diflubenzuron and 2,6-difluorobenzamide were found and the remainder was conjugates (about 18.7 percent).

In another study of fate of diflubenzuron, Still and Leopold (1978) treated boll weevils (ebony strain) by immersion, injection and feeding of the radiolabeled (^{14}C) compound. Immersion was into 12 ng/μl of radiolabeled diflubenzuron; injections were of 66.3 ng [^{14}C]/weevil; and feeding was on cotton squares, about 120 ng [^{14}C] for 66 hours.

After 13 days, both the immersed and injected weevils showed less than 10 percent ^{14}C concentration; the remainder of the ^{14}C was found in the frass. Forty-four hours after removing the fed insects, 50 percent of the radiolabel had been excreted as unchanged diflubenzuron.

Bull (1980) reports on effects of diflubenzuron on hatchability of eggs of the boll weevil. Female boll weevils received topical applications of radiolabeled diflubenzuron. Ten days after treatment, hatch was inhibited by about 80 percent; at 22 days hatch increased to a level of 80 to 90 percent. When 50 percent of the eggs had hatched, about 16 days after treatment, the diflubenzuron content was about 0.6 ppm.

Truchet et al. (1981) examined the effect of exposure of Pieris brassicae (Lepidoptera) larva (first 3 days of 5th instar) to a concentration of 300 ppm diflubenzuron over a 3-day period. Because of their involvement in diflubenzuron action, integument and fat bodies were analyzed by ion microanalysis. Flouride intensities were measured to detect action of the diflubenzuron. In treated larva, diflubenzuron was diffused to the whole tissues, not just to target cells (the epidermal cells, which synthesize the chitin) and was particularly concentrated in perinuclear protein granules of visceral fat body.

Metabolism of diflubenzuron in the two-spotted spider mite (T. urticae) is reported in Franklin and Knowles (1980). The mites were exposed to radiolabeled diflubenzuron (^{14}C) via a metabolism chamber, for exposure periods of 1, 12, 24, 48, 72, and 96 hours. During the 96 hours, less than 10 percent of the radiocarbon was detected in the insects. Metabolism of absorbed diflubenzuron proceeded at a slow rate; the compound decreased from 97.4 percent at 1 hour to 69.8 percent after 96 hours.

Seuferer et al. (1979) describes the metabolic fate of diflubenzuron in four genera of soil fungi: Fusarium, Cephalosporium, Penicillium, and Rhodotorula. The fungi were cultured in medium containing 5 mg diflubenzuron and transferred weekly for 10 weeks. The fungi that broke down diflubenzuron most actively were Fusarium spp, the only genus of the four to use the compound as its main carbon source. Half-lives of detectable diflubenzuron ranged from 7 (Fusarium) to 18 (Rhodotorula) days. Control (uninoculated) medium had a half-life of detectable diflubenzuron of 21 days. Metabolites detected in the Fusarium culture medium included 2,6-difluorobenzoic acid and 4-chlorophenylurea, which was further metabolized to 4-chloroaniline. Only the first two metabolites were detected in the three remaining species after 30 days. Further experiments demonstrated that 2,6-diflurobenzoic acid was lethal to Salmonella.

Mayer et al. (1984) examined diflubenzuron inhibition of nucleoside uptake in Harding-Passey melanoma cells. Diflubenzuron solution (1, 10, and 25 μM diflubenzuron plus dimethyl sulfoxide) was added at 1 μl/ml medium to cell cultures. Significant inhibition of uptake of uridine, adenosine, and cytidine (but not thymidine) occurred very rapidly (i.e., ≤5 minutes). Inhibition of uptake of nucleosides was not reversible.

3.2 Soil

Studies have indicated that diflubenzuron is not persistent nor very mobile in soil. The short half-life of a few days to 1 week appears to be unrelated to the type of soil but is dependent on microbial activity in the soil and the particle size of diflubenzuron (Verloop and Ferrel 1977, Seuferer et al. 1979, Nimmo et al. 1984 all in Dobrowski et al. 1985). After incorporating diflubenzuron into the top 7.6 cm of agricultural soil at an application rate of 0.13 kg a.i./ha (0.3 ppm), the half-life of diflubenzuron was less than 48 hours. After 4 weeks, only 0.003 ppm leached into the 7.6 to 15.2 cm layer of soil (White 1975, in Dobrowski et al. 1985). In a study of residues in forest litter and soil (Sundaram 1987, Sundaram et al. 1987), diflubenzuron concentrations decreased from 0.46 ppm to less than 0.1 ppm within 5 days. Jones and Kochenderfer (1986) reported residues decreasing from 2 parts-per-billion to less than 30 parts-per-trillion within 3 hours after application.

With respect to particle size, Marx (1977, in Dobrowski et al. 1985) reported that experiments on the half-life of diflubenzuron in soil showed that smaller diflubenzuron particle size was directly related to shorter half-life of the pesticide. By reducing the average particle size from 10 to 2 μm, the net half-life was reduced from 16 weeks to less than 1 week.

Duphar (1985) and Nimmo et al. (1984) indicate that diflubenzuron is readily degraded in soils of various types under both aerobic and anaerobic conditions. Fifty percent degradation is reached in 2 to 6 days, if the chemical is applied to the soil as an aqueous dispersion of particles about 2 μm in size at a dose of 1 mg/kg (average field dose and particle size available as dispersible powder).

Duphar reports that the primary soil metabolites are 4-chlorophenylurea and 2,6-difluorobenzoic acid. The acid is unstable and decomposes by decarboxylation and ring cleavage by 50 percent in 1 to 2 weeks. The 4-chlorophenylurea is metabolized by 50 percent in soil in about 5 weeks, gradually converting into bound residuals. Apparently, the breakdown of 4-chlorophenylurea in soil is a complex process in which 4-chlorophenylurea may be an intermediate compound (yet to be detected in soil).

The U.S. distributor of diflubenzuron (Uniroyal 1983) indicates that the half-life of Dimilin in soil is strongly dependent upon the particle size of diflubenzuron as applied. With an average particle size of about 2 μm, the half-life is between 3 and 7 days. A particle size of 10 μm results in a half-life of from 8 to 16 weeks. Evidence of metabolic action in the formation of the two main metabolites is that degradation in steam sterilized soil is extremely slow (indicating microbial action).

Dobrowski et al. (1985) report that the main degradation pathway in soils for more than 90 percent of diflubenzuron is hydrolysis. The degradation products are 2,6-difluorobenzoic acid, 4-chlorophenylurea, and 4-chloroniline. The last compound is a minor degradation product. Although agreeing with the manufacturer that the half-life of 2,6-difluorobenzoic acid is 1 to 2 weeks, Dobrowski cites evidence that 4-chlorophenylurea is converted into bound residues with a half-life of 5 to 10 weeks. Free 4-chloroaniline has not been found, presumably due to rapid binding of diflubenzuron to the soil (Dobrowski et al. 1985, in Maas et al. 1980).

Dobrowski et al. (1985) caution that the fate of diflubenzuron in soil should be distinguished between diflubenzuron introduced directly to the soil (resulting in a short half-life) and diflubenzuron introduced into the soil when it is absorbed in litter and in plants milled and cultivated into the soil. The authors cited a study (Bull and Ivie 1978) that followed the fate of diflubenzuron repeatedly applied ten times to a field of cotton. The pesticide entered the soil as runoff of sprays (direct entry) and when treated plants were milled and cultivated into the soil (indirect entry). There was no appreciable decline in overall residue concentrations for almost 9 months after the first application. However, 3 months later, soil residues had declined from 0.3 to 0.13 mg/kg. Probably the actual composition of the residue is a complex of metabolic conversion products due to the very short half-life of diflubenzuron in soil. The uptake of soil residues by agricultural crops rotated in place of the treated cotton was minimal. Edible parts of these rotational crops were unaffected.

3.3 Water

Dobrowski et al. (1985) reviewed the literature on the fate of diflubenzuron in water. On the basis of their review, the authors concluded that diflubenzuron degrades rapidly in neutral or alkaline waters and that, without considering its potential biodegradation in natural waters, the pesticide has the potential for persistence or accumulation in water only under quite acidic conditions. Because the pH of most natural waters is between 6 and 9, the use of diflubenzuron as an insect control agent should not normally result in persistent residues in natural waters. In the aquatic environment, the half-life of diflubenzuron is related to pH, water temperature, amount of suspended organic particles in water, and subsequent microbial degradation. A study of shallow pond ecosystems (Kingsbury et al. 1987) reported that it had a water half-life of 0.4 to 1.4 days and reached nondetectable levels in pond sediment after 3 to 4 days.

Ivie et al. (1980, in Dobrowski et al. 1985) reported that diflubenzuron degrades in an aqueous environment through predictable hydrolytic mechanisms and, to a lesser extent, by cyclization. The major breakdown products from nonbiological hydrolysis are the same as the three reported above for hydrolysis in the soil environment. Dobrowski (1985) reports that the results of several studies indicate that hydrolysis to 4-chlorophenylurea is the primary means of degradation.

When exposed to sunlight, the stability of diflubenzuron in water is relatively short. Data from exposure studies conducted by Schaefer and Dupras (1979) indicate that the photolysis half-life of diflubenzuron is about 81 hours (3.4 days).

The degradation to 4-chloroaniline in natural waters may represent a health concern because this compound has been classified as a mutagen by the National Cancer Institute and the Cancer Assessment Group of the U.S. Environmental Protection Agency. On the other hand, the World Health Organization and the Food and Agriculture Organization of the United Nations have determined that 4-chloroaniline is a nonmutagenic, transient metabolite based on results from in vitro and in vivo studies (Food and Agriculture Organization 1981). As within the soil environment, 4-chloroaniline is a minor degradation product.

In the marine environment, diflubenzuron is relatively nonpersistent. Schimmel et al. (1983) reported a half-life for diflubenzuron in seawater to be less than 4 days.

Christiansen and Costlow (1980, in Dobrowski et al. 1985) studied the persistence of diflubenzuron in filtered estuarine water having a pH of 6.5 and a salinity of 20 parts-per-trillion. Crab larvae (*Rhithropanopeus harrisii*) were used as test organisms. Within the tests, no larvae survived to the first crab stage until the diflubenzuron suspensions had aged for 42 days. It required an additional 14 to 17 days before there was no apparent affect of the larvae. Although the concentration of diflubenzuron was not measured during the tests, the tests indicated that some estuarine species are sensitive to concentrations of diflubenzuron the low parts-per-billion range.

3.4 Air

Diflubenzuron has a relatively low vapor pressure at environmental temperatures, less than 1.3×10^{-5} Pa at 50 °C (Duphar 1985). Thus, diflubenzuron should not vaporize readily into the atmosphere.

Schaefer and Dupras (1979) studied the stability of diflubenzuron on glass surfaces exposed to direct sunlight. The authors determined that diflubenzuron has a half-life of about 46 hours under the test conditions. This half-life was calculated and extrapolated from results of exposure periods ranging from 0 to 9 hours.

After conducting a literature survey, Dobrowski et al. (1985) did not find reports of studies on the fate of diflubenzuron in the atmosphere.

4.0 Exposure and Hazard Assessment

Forest workers, residents, visitors, and transient animals are all subject to potential exposure to diflubenzuron from forest aerial applications. For both animals and humans, the most extreme exposures are expected to be directly associated with the application process and can be by dermal, oral, or inhalation routes. Fish and aquatic insects take up diflubenzuron from water and sediments but bioaccumulation generally does not exceed 100 times ambient concentration and is reversible after removal of exposure. Diflubenzuron is readily degradable in the environment, has a half-life of less than or equal to 1 week in soil and water, and exhibits virtually no mobility in soil (Verloop and Ferrel 1977, Seuferer et al. 1979, Ivie et al. 1980). The following discussion is based upon the risk analysis performed by the USDA Forest Service (1985) in a Supplemental Draft Environmental Impact Statement (EIS) on Gypsy Moth Suppression and Eradication Projects.

Diflubenzuron is reported to be nonmutagenic (MacGregor et al. 1979, U.S. EPA 1984, in USDA Forest Service 1985) and noncarcinogenic (Quarles 1980, Patel and Santolucito 1980, in USDA Forest Service 1985). When diflubenzuron was first registered, the EPA's Carcinogen Assessment Group reported suggestive evidence that it may be carcinogenic in female mice. However, subsequent carcinogenicity studies in mice and rats completed and reviewed by EPA showed that diflubenzuron was noncarcinogenic at all doses tested (USDA Forest Service 1985). Epidemiological studies are lacking for cancer, birth defects, or systemic effects.

Exposure studies are lacking for both workers and the general public. However, estimates of realistic and worst-case doses are more than two orders of magnitude less than the lowest NOEL value for diflubenzuron. All but one of the realistic and worst-case expected occupational doses are several fold less than the Acceptable Daily Intake (ADI) level set by EPA and that worst-case dose estimate equals the ADI. It is highly unlikely that humans or animals, large or small, would be adversely affected at the realistic or worst-case dose ranges, which are very conservative (overestimates).

4.1 USDA Forest Service Application

The USDA Forest Service uses diflubenzuron as a spray from the ground as well as aerially for gypsy moth eradication. Diflubenzuron is also used commercially as either a 25 percent wettable powder, a 48 percent oil dispersible flowable concentrate, or a granule. Granules are used in field formulation for mosquito control. For gypsy moths, the USDA Forest Service used diflubenzuron on 50 to 8,991 acres/year from 1983 to 1987 (Gross 1987). Typically, workers involved in a spray program would be mixers/loaders and observers, including pilots.

4.2 Exposure

The USDA Forest Service (1985) estimated a "generic" single application of 1 lb a.i./acre applied in 1 gallon of total formulation. It also assumed that such insecticides are applied by aircraft using 100 to 150 μm droplets to minimize inhalation potential and drift. The worst-case exposures to diflubenzuron are anticipated to occur in workers who come into contact with the powdered pesticide during the mixing and loading process. Dermal and inhalation exposures are expected under such circumstances. Exposures to observers, including pilots, were estimated by the USDA Forest Service (1985) to be about one third that received by the mixers/loaders. All other occupational exposures of project personnel were assumed to be equal to that of the highest exposure potential group, mixers/loaders. An average of 15 persons (jobs unspecified) are exposed in a USDA Forest Service Gypsy Moth control program using diflubenzuron. Exposure is usually 6 hours/day and usually occurs at a frequency of 6 days/year (Gross 1988).

In addition, indirect exposure to humans occur to residents and others traveling through the site of application. Indirect exposures also occur when animals and/or vegetation taken from a treated area are ingested (oral exposure). Goats, rabbits, and fish were used as models by the USDA Forest Service (1985) to estimate potential exposure from eating game and domestic animals from treated areas.

Accidental exposure due to truck or airplane spills also would result in worst-case doses. Truck spill scenarios present the greatest human health hazard but have the lowest probability of occurring. The USDA Forest Service (1985) risk analysis model calculates a probability of occurrence of 1.08×10^{-5} on land or 1 spill on land for every 93,000 vehicle trips and 1.2×10^{-6} in water or 1 spill into water for every 833,000 vehicle trips (realistic scenario). The probability of a worst-case spill on land is 1.2×10^{-6} or 1 worst-case spill on land/50 million trips and the probability of such a spill on water is 2.2×10^{-9} or 1 worst-case spill in water/500 million trips. Worst-case doses are estimated to be 1 chance in 555.

The USDA Forest Service (1985) calculated probabilities for "realistic" aircraft spills are 5.1×10^{-4} on land (or 1 spill for every 1,960 aircraft loads) and 5.7×10^{-5} in water (or 1 spill for every 17,554 aircraft loads). For worst-case spills, the probabilities dropped to 9.1×10^{-7} on land (or 1 spill for every 1,000,000 aircraft loads) and 1.0×10^{-9} in water (or 1 spill for every 100 million aircraft loads).

4.2.1 Occupational Dose

Inhalation doses are expected to be minimal (0.2 percent of exposure) and are, therefore, incorporated into estimates of dermal doses. For the purpose of risk analysis, a 10 percent dermal absorption value was used by USDA Forest Service (1985) to derive these estimates.

For mixers/loaders, the realistic and worst-case dermal dose estimates were 0.042 mg/kg/day and 0.1 mg/kg/day, respectively. For observers, the realistic and worst-case dermal dose estimates were 0.002 mg/kg/day and 0.029 mg/kg/day, respectively.

To calculate the expected dose level for diflubenzuron, the realistic and worst-case dose levels are multiplied by the maximum registered application rate of 0.06 and either the realistic application error factor of 1.1 or the worst-cast application error factor of 2. For example, for mixers/loaders, the expected realistic dose is 0.0028 mg/kg/day (0.042 mg/kg/day x 0.06 x 1.1) and the worst case expected dose is 0.012 mg/kg/day (0.1 mg/kg/day x 0.06 x 2). Expected dermal and inhalation doses to diflubenzuron are presented in table 4-1.

4.2.2 Environmental Exposure

Humans are exposed environmentally when forest visitors or others not directly involved in spray operations come in contact with spray or sprayed foliage, inhale spray mist, eat plants or animals that are contaminated, or drink water containing diflubenzuron. Animals, both terrestrial and aquatic, are subject to environmental exposures as well.

4.2.2.1 Dermal Exposures. Human dermal environmental exposures would be less than occupational exposures because only workers would be directly involved with actual activities in treatment areas. Residents and visitors to treatment areas would be expected to receive an exposure that is orders of magnitude less than that of the mixer/loader or backpack sprayer. For residents, the USDA Forest Service (1985) realistic and worst-case dose estimates were 0.002 mg/kg/day and 0.004 mg/kg/day, respectively, assuming 8 hours of exposure to drift and a 10 percent dermal absorption rate. However, residents could receive doses as high as observers if they were subjected to direct application.

In addition, persons within the drift of the application site may be exposed to insecticides by dermal contact, inhalation, or ingestion. However, dermal and inhalation exposures of persons downwind are low relative to those experienced by project personnel. Witt (1984) reviewed a number of drift studies (which referred to 75 to 100 mm drop size) and reported drift values of 2, 0.5, and 0.15 percent of the amount deposited on targets at distances from the application site of 1/4, 1/2, and 1 mile,

Table 4-1

Expected Dose Levels for Diflubenzuron

	Realistic (mg/kg/day)	Worst Case (mg/kg/day)
Occupational		
Mixers/Loaders	0.0028	0.012
Observers	0.00013	0.0035
Environmental		
Direct Exposure	0.00013	0.00048
Near Range Drift	0.000087	0.00032
Indirect Exposure	0.000026	0.00011

respectively. These values are used for calculating doses received from insecticidal drift because they are based on actual drift data, and involve droplet sizes typical of gypsy moth spray programs. Extrapolation of these data for near range drift (250 feet from the treatment area) shows the dose to be approximately 67 percent of that for a resident in the treatment area. For the USDA analysis then, the realistic and worst-case doses for near range drift is calculated to be 0.0013 mg/kg (0.002 x 0.67), respectively (USDA Forest Service 1985).

The expected human environmental dermal dose levels after correction for application rate and application error (see section 4.2.1) are presented in table 4-1. The expected realistic dose level for direct exposure is 0.00013 mg/kg/day (0.002 x 0.06 x 1.1) for direct exposure of residents and 0.000087 mg/kg/day (0.0013 x 0.06 x 1.1) for near drift exposure.

Animals in the target spray zone are also subject to dermal exposure from aerial application of diflubenzuron. It was assumed that animals would not be sprayed directly by backpack applicators and that the animals' ventral surfaces would contact foliage that had been sprayed with liquid formulations. Thus, approximately half of the animals' body surface would contact sprayed vegetation, resulting in an exposure level equal to half of the exposure that would be calculated for aerial application methods at the maximum rate of application (aerial application amounts for exposure of the dorsal surface as well as ventral). Rabbits and goats, representing small and large game animals, respectively, have the following estimated dermal exposures (USDA Forest Service 1985).

Dermal exposure and dose estimates for goats assume the following:

- The goat weighs 40 kg (88 lbs) and has 2.3 m^2 surface area.
- The dorsal surface is contacted by deposited spray.
- Treated foliage contacts the goat's sides and stomach as the animal walks through it, at a concentration equivalent to direct deposition.
- Dermal absorption rate ranges from 1 to 10 percent. A 10 percent absorption rate is considered to be worst-case assumption because dermal toxicity studies are conducted on shaved animals. Animal hair acts like human clothing protecting against dermal exposure.

The estimated dermal exposure of a goat is 2.02 mg/kg (35 mg/m^2 x 2.3 m^2/goat x goat/40 kg) for each 1 lb a.i. applied/acre. The estimated dermal dose to the goat ranges from 0.020 to 0.202 mg/kg (2.02 mg/kg x 0.01 or 0.1) depending on the dermal absorption rate.

Dermal exposure and dose estimates for rabbits assume the following:

- The rabbit weighs 2.0 kg and has 0.23 m^2 surface area.
- The dorsal surface is contacted by deposited spray.
- The ventral surface is contacted by treated foliage as the animal walks through it, with an insecticide concentration equivalent to direct deposition.
- Dermal absorption rate ranges from 1 to 10 percent, with the latter rate considered a worst-case assumption for reasons previously stated.

The estimate dermal exposure to rabbits is 4.03 mg/kg (35 mg/m^2 x 0.23 m^2 rabbit x rabbit/2 kg) for each 1 lb a.i. applied/acre. The estimated dermal dose to rabbits ranges from 0.040 to 0.403 mg/kg (4.03 mg/kg x 0.01 or 0.1).

4.2.2.2 Inhalation Exposures. Human inhalation environmental exposures would be less than occupational exposures since spray operators, involved with activities on the spray units, are more likely to be subject to spray mist than is a casual visitor. Thus, a casual forest visitor should be expected to receive an inhalation exposure that is orders of magnitude less than that received by a backpack sprayer. Environmental inhalation exposures of animals in the spray target area would occur on a one-time basis and would be limited to a time period that can be measured in minutes following application of liquid formulations. Inhalation of diflubenzuron following its application would be negligible. Inhalation exposure is, therefore, expected to be so small (0.2 percent of exposure) that it can be included with dermal exposure estimates.

4.2.2.3 Immersion Exposures. Aquatic organisms are subject to environmental exposures to diflubenzuron in contaminated streams and ponds that are adjacent to target spray zones. Environmental exposures for aquatic organisms are expected to be negligible, about 0.05 mg/kg/day, a deliberate overestimate (USDA Forest Service 1985). It is also important to note that diflubenzuron is rapidly degraded in neutral or alkaline water. Its half-life in natural waters, pH 6 to 9, is less than 1 week. Bioaccumulation of diflubenzuron does not generally exceed 100 times ambient levels and is reversible after removal of exposure.

4.2.2.4 Oral Exposures. Oral environmental exposure could result from wildlife eating sprayed foliage or from human consumption of large or

4.2.1 Occupational Dose

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Dermal exposure and dose estimates for goats assume the following:

- The goat weighs 40 kg (88 lbs) and has 2.3 m^2 surface area.
- The dorsal surface is contacted by deposited spray.
- Treated foliage contacts the goat's sides and stomach as the animal walks through it, at a concentration equivalent to direct deposition.
- Dermal absorption rate ranges from 1 to 10 percent. A 10 percent absorption rate is considered to be worst-case assumption because dermal toxicity studies are conducted on shaved animals. Animal hair acts like human clothing protecting against dermal exposure.

The estimated dermal exposure of a goat is 2.02 mg/kg (35 mg/m^2 x 2.3 m^2/goat x goat/40 kg) for each 1 lb a.i. applied/acre. The estimated dermal dose to the goat ranges from 0.020 to 0.202 mg/kg (2.02 mg/kg x 0.01 or 0.1) depending on the dermal absorption rate.

Dermal exposure and dose estimates for rabbits assume the following:

- The rabbit weighs 2.0 kg and has 0.23 m^2 surface area.
- The dorsal surface is contacted by deposited spray.
- The ventral surface is contacted by treated foliage as the animal walks through it, with an insecticide concentration equivalent to direct deposition.
- Dermal absorption rate ranges from 1 to 10 percent, with the latter rate considered a worst-case assumption for reasons previously stated.

The estimate dermal exposure to rabbits is 4.03 mg/kg (35 mg/m^2 x 0.23 m^2 rabbit x rabbit/2 kg) for each 1 lb a.i. applied/acre. The estimated dermal dose to rabbits ranges from 0.040 to 0.403 mg/kg (4.03 mg/kg x 0.01 or 0.1).

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4.2.2.4 Oral Exposures. Oral environmental exposure could result from wildlife eating sprayed foliage or from human consumption of large or small animals or fish. Both humans and animals could drink contaminated water, which could present a significant exposure, for example, within a day or two of a spill of diflubenzuron into open water.

Oral exposure and dose estimates for goats and rabbits assume the following:

- Post-spray concentration insecticides in browse and food foliage ranges from 10 ppm (realistic) to 100 ppm (worst case).

- A typical goat weighs 40 kg (88 lbs) and eats 1.15 kg (2.5 lbs) field weight foliage/day. A typical rabbit weights 2.0 kg, eats 60 g of food (dry weight) daily, and receives a dose of 0.03 mg/kg/day for each ppm in food (USDA Forest Services 1985).

- Oral dose equals oral exposure.

Thus, the realistic and worst-case exposures for a goat due to ingestion would be 0.29 mg/kg/day (10 mg/kg x 1.15 kg/goat/day x goat/40 kg and 2.88 mg/kg/day (100 mg/kg x 1.15 kg/goat/day x goat/40 kg) for each 1 lb a.i./acre applied. Estimated doses are the same (0.29 and 2.88 mg/kg/day, respectively) (USDA Forest Service 1985).

Similarly, a typical rabbit would have realistic and worst-case exposures of 0.3 mg/kg/day (10 ppm x 0.03 mg/kg/day/ppm) and 3.0 mg/kg/day (100 ppm x 0.03 mg/kg/day/ppm) for each 1 lb a.i./acre applied. Estimated doses are the same (0.03 and 3.0 mg/kg/day), respectively (USDA Forest Service 1985).

For each pound of diflubenzuron applied, the realistic dose received from a daily diet of 0.5 kg of meat (or fish), 0.5 kg of vegetables and fruits, and 2 liters of water has been estimated to be 0.0089 mg/kg/day (0.004 + 0.0071 + 0.0014 mg/kg/day; see table 4-2). The worst-case dose received from such a diet has been estimated to be 0.061 mg/kg/day for diflubenzuron (USDA Forest Service 1985). The probability of occurrence of a worst-case diet was estimated to be 1.8×10^{-3} or 1 chance in 500. When these estimates are corrected for application rate and application error, the expected realistic dose is 0.00059 mg/kg/day and the expected worst-case dose is 0.0073 mg/kg/day.

4.3 Hazard Assessment

A summary of expected (realistic) occupational dose, environmental exposures (realistic), and toxicity values for diflubenzuron are presented in table 4-2. Diflubenzuron is not very persistent in the environment, soil or water. It is virtually immobile in soil and is not expected to leach into groundwater. Diflubenzuron is bioaccumulated by aquatic organisms but the process is reversible when exposure is removed. In

Table 4-2

Summary of Occupational Dose, Environmental Exposure and Toxicity for Diflubenzuron

	Exposure (mg/kg/day)[a]	
	Dermal/Inhalation[b]	Oral
Occupational Dose		
Mixer/loader	0.0028	No dose calculated
Observer	0.00013	No dose calculated
Environmental Exposure		
Fish[c]	0.05	0.05
Large animal (goat)	0.02	0.29
Small animal (rabbit)	0.04	0.30
Humans		
Direct exposure	0.00013	NA
Near drift	0.000087	
Ingestion		
Fish	NA	0.0004
Rabbit	NA	0.0000001
Goat	NA	0.000002
Water	NA	0.0014
Vegetables and Fruits	NA	0.007
Toxicity Summary	Dermal LD_{50} >4,000 mg/kg	LD_{50} >40,000 mg/kg
	Inhalation LC_{50} >35 mg/l	NOEL - 40 to 160 mg/kg
	For fish, LC_{50} >100 mg/l; practically nontoxic.	

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[a] Assumes aerial application fine droplets 1 lb a.i./acre diflubenzuron (USDA Forest Service 1985); realistic doses given; bioconcentration of 1.0 in fish.

[b] Included with dermal exposure, inhalation only 0.2 percent of exposure.

[c] Fish exposure deliberate overestimate from immersion exposure to about 50 ppb; bioconcentration of 1.0.

NA - nonapplicable data.

addition, diflubenzuron is practically nontoxic to fish with an LC_{50} greater than 100 mg/l. Concern has been raised that this chitin synthesis inhibitor may have adverse effects on crustaceans (Costlow 1979, Nimmo et al. 1980, Tester and Costlow 1981, in Weis and Ma 1987). However, a study by Weis and Ma (1987) indicates that the larvae of horseshoe crabs (Limulus polyphemus), are quite resistant.

While diflubenzuron is very toxic to target pest species, it is only slightly toxic to mammals. It is nonmutagenic and noncarcinogenic and has very high dermal and oral LD_{50} values, >4,000 mg/kg and >40,000 mg/kg, respectively. The NOEL for systemic effects for diflubenzuron is 40 mg/kg/day and the NOEL for systemic toxicity for diflubenzuron in a reproductive study is 160 mg/kg/day, the highest dose tested. According to the USDA Forest Service (1985), the lowest NOEL for teratogenic effects following dosing with diflubenzuron is 4,000 mg/kg/day.

A joint meeting in Geneva on 23 September to 2 October 1985 of the Food and Agriculture Organization of the United Nations and a panel of experts on pesticide residues resulted in the setting of an ADI for diflubenzuron of 0.02 mg/kg/day (FAO 1985). The EPA established an ADI for diflubenzuron of 0.011 mg/kg/day.

The expected occupational and environmental dermal and inhalation doses of diflubenzuron are more than two orders of magnitude less than the experimentally determined NOEL levels in animals. In addition, only expected occupational doses and the worst-case dietary dose approach the ADI established by the EPA and none exceed it. Based on its low toxicity and assuming that accident scenarios will generally not exceed worst-case occupational exposures, there appears to be no great threat from accidental releases of diflubenzuron. Similarly, estimates of large and small animal exposures are many orders of magnitude below LD_{50} values and NOEL levels. It seems highly unlikely that humans or animals will be adversely affected by diflubenzuron use. However, care should be taken during the use of diflubenzuron because data regarding human exposure and toxicity are lacking.

Appendix A

Summary of Diflubenzuron Toxicity Data for Microorganisms

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Summary of Diflubenzuron Toxicity Data for Microorganisms

Formulation	Organism	Nature of Exposure	Time	Effects	Comments	Reference
Diflubenzuron	Diatoms	Static chronic	120 days	No effect ≤45 μg/l	NA	Hanson and Garton 1982
Diflubenzuron	Soil fungi (*Fusarium*, *Cephalosporium*, *Penicillium*, *Rhodotorula*) and *Salmonella* bacteria	*in vitro* incubation	6 to 20 days	Rapid degradation by 4 eukaryotic microbes; diflubenzuron metabolites lethal to *Salmonella*.	Nontarget	Seuferer et al. 1979
Diflubenzuron and 4-chlorophenyl urea, 2,6 difluorobenzoic acid and 4-chloroaniline	*Euglena gracilis* cells	In glycine $U\text{-}C^{14}$ in protein culture	30 hrs	4-chloroaniline caused considerable inhibition to growth but other metabolites did not inhibit protein biosynthesis.	Nontarget	Gattavecchia et al. 1981

NA - data not available.

Appendix B

Summary of Diflubenzuron Toxicity Data for Invertebrates

Appendix B

Summary of Diflubenzuron Toxicity Data for Invertebrates

Formulation	Organism	Nature of Exposure	Time	Effects	Comments	Reference
Diflubenzuron 95% technical	G. pseudolimnaeus	95% CI (mg/l) acute immersion	96 hrs	LC_{50} = 0.030 (0.021-0.043)	12 °C temp.	Johnson and Finley 1980
25% WP	Daphnia magna	95% CI (mg/l) acute immersion	48 hrs	EC_{50} = 0.016 (0.012-0.020)	Immature, 22 °C	Johnson and Finley 1980
	G. pseudolimnaeus	95% CI (mg/l) acute immersion	96 hrs	LC_{50} = 0.025 (0.016-0.040)	12 °C temp.	Johnson and Finley 1980
	Chironomus	95% CI (mg/l)	48 hrs	EC_{50} = 0.56 (0.47-0.67)	Immature, 22 °C	Johnson and Finley 1980
Dimilin	(Musca domestica) fly larvae	Contaminated soil 75 g a.i./ha	Immediate	12% of larvae died	Aerial application forest cover	Barth 1981
	(Musca domestica) fly larvae	Contaminated soil 75 g a.i./ha	Immediate	100% mortality	Laboratory	Barth 1981
Dimilin 25 WP	Bees	0.24 kg/ha	NA	Not toxic at lowest rate.	--	Wilkaniec 1980
Dimilin 25% WP	Mosquitoes Aedes nigromaculis A. melanimon	4 applications in field 0.02 lb a.i./acre and 0.04 lb a.i./acre	56 days	Not detectible after 3 to 4 days (0.02 lb/acre) or 7 days (0.04 lb/acre) excellent for mosquito control, 100% for larvae.	Aerial application	Schaefer and Dupras 1977
Difluron 25% WP and technical	Western spruce budworm (Choristoneura occidentalis)	1 µl/100 mg to thoracic dorsa 3 times topical	NA	No toxicity when applied topically except 25 WP activity against 4th instar tussock moth (LD_{50} = 70 µg/g); in diet LC_{50} = 0.028 ppm (0.018-0.040) for 25 WP.	Diet results influenced by solvent used.	Gillette et al. 1978
	Douglas-fir tussock moth (Orgyia pseudotsugata)	8 concentrations fed 4 times	NA			

Appendix B (Continued)

Formulation	Organism	Nature of Exposure	Time	Effects	Comments	Reference
Dimilin 25% WP	Honey bee (*Apis mellifera*)	350 g/ha spray	8 days	No effects on eggs or larvae or honey production.	--	Buckner et al. 1978
Dimilin 25% WP	Juvenile crayfish (*Orconectes virilis*)	1, 10, 100 and 1000 ppm aqueous solutions	2 wks	No toxic effects up to 500 ppm.	Problems with lab procedure.	Buckner et al. 1975
Dimilin 25% WP	Snails, amphipods, beetles and 10 other orders of bottom fauna	350 g/ha spray over streams	10 days	No effects on total numbers but reduced amphipod and aquatic beetle larvae populations.	--	Buckner et al. 1975
Diflubenzuron 25% WP	Honey bee (*A. mellifera*)	100 ppm a.i. in water and sucrose (syrup 80 ppm)	40 days	Produced less brood, 42% survived; area and weights of brood significantly reduced.	Difference in survival not significant.	Barker and Waller 1978
TH 6040	Water flea, copepods, and shrimp	0.05 ppm in static aquaria	30 days 24 hrs for LC_{50}	Water flea population markedly reduced 3 days but recovered, reproduction suspended 1 week; copepods small reduction; shrimp no harmful effect.	LC_{50} (ppm) water flea = 1.8×10^{-3} seed shrimp = 5 copepod = 10	Miura and Takahashi 1974
Th 6040	*Triops longicaudatus*, *Eulimnadia* spp. *Daphnia*, *Moina* spp.	Laboratory, ponds and irrigation 0.005 ppm or rate in field 0.1 lb a.i./acre	2 mos	Few crustaceans are susceptible to TH 6040 at levels below 0.01 ppm; shrimp disappeared in 3 days.	Nontarget aquatic species	Miura and Takahashi 1974
	Cyclops, *Diaptomus*	Laboratory, ponds and irrigation 0.005 ppm or rate in field 0.1 lb a.i./acre	2 mos	Some tolerance at 0.01 ppm	Nontarget aquatic species	Miura and Takahashi 1974
	Cypricercus, *Cypridopsis*			Tolerant to 0.5 ppm	Nontarget aquatic species	Miura and Takahashi 1974
	Callibaetis, *Goeldichironomus holoprasinus*	Laboratory, ponds and irrigation 0.005 ppm or rate in field 0.1 lb a.i./acre	2 mos	In sensitive species, adult emergence was affected.	Nontarget aquatic species	Miura and Takahashi 1974

Appendix B (Continued)

Formulation	Organism	Nature of Exposure	Time	Effects	Comments	Reference
TM 6040	Thermonectus basillaris, Laccophilus, Hydrophilus triangularis, Tropisternus lateralis, Hyalla azteca, Notonecta unifasciata	Laboratory, ponds and irrigation 0.005 ppm or rate in field 0.1 lb a.i./acre.	2 mos	Strong tolerance	Nontarget aquatic species	Miura and Takahashi 1974
		Laboratory, ponds and irrigation 0.005 ppm or rate in field 0.1 lb a.i./acre.	2 mos	Suppressed reproduction	Nontarget aquatic species	Miura and Takahashi 1974
Diflubenzuron	Buenoa spp., Trichocorixa louisianae, Coenagronidae naiad, Berosus infuscatus, Hyalella azteca	28 g a.i./ha treatment in marsh	18 mos	Significant (p <0.01) reduction of population.	Nontarget aquatic species	Farlow et al. 1978
				Significant reduction		
				Significant (p <0.01) reduction in population. Significant reduction.	Nontarget aquatic species	Farlow et al. 1978
	15 other taxa	28 g a.i./ha treatment in marsh	--	Significant (p <0.05) increase in population.	Nontarget aquatic species	Farlow et al. 1978
	27 other taxa	28 g a.i./ha treatment in marsh		No differences following treatment.	Nontarget aquatic species	Farlow et al. 1978
Dimilin (TM 6040)	Brine shrimp (Artemia salina)	1, 2, 5, 10 ppb in ocean water nauplii exposed >10 ppb; zygotes fed	--	Significant reduction in reproductive lifespan >2 ppb; lower hatchability; nauplii exposed >10 ppb died within 3 days, but no difference in 1 to 10 ppb groups.	Nontarget aquatic species	Cunningham 1976
Diflubenzuron (technical)	Barnacle Balanus eburneus	750 to 1,000 ppb in seawater	10 days	Delayed cuticle secretions in premolt phase.	Nontarget aquatic species	Gulke et al. 1982
Diflubenzuron	Midge (Cricotopus sp.),	Acute flow through	7 days	LC_{50} = 1.79 μg/l	Nontarget	Hansen and Garton 1982
	(Tanytarsus dissimilis),	Acute flow through	5 days	LC_{50} = 1.02 μg/l	Nontarget	Hansen and Garton 1982
	Gammarid (Hyallela azteca),	Acute flow through	96 hrs	LC_{50} = 1.84 μg/l		
	Daphnia magna	Acute static Chronic.	48 hrs 21 days	Acute LC_{50} = 4.4-6.9 μg/l Chronic LC_{50} = 0.062 μg/l survival and reproduction	Nontarget Nontarget	Hansen and Garton 1982

Appendix R (Continued)

Formulation	Organism	Nature of Exposure	Time	Effects	Comments	Reference
Diflubenzuron	Snail Juga plicifera	Acute flow through Chronic	96 hrs 2 wks	No effect ≤45 μg/l No effect ≤45 μg/l	Nontarget	Hansen and Garton 1982
	Physa sp.	Acute flow through Chronic	7 days 2 wks	No effect ≤45 μg/l No effect ≤45 μg/l	Nontarget	Hansen and Garton 1982
Diflubenzuron (Dimilin) 25% WP 95% technical	Spodoptera littoralis larvae	Dietary on castor bean leaf and topically	2 days	Sterilized adults emerging Resistent strain developed; EC_{50} = 3 ppm 3rd instar and EC_{50} = 15 ppm 5th instar ED_{50} = 0.073 μg/larvae 3rd instar ED_{50} = 0.08 μg/larvae 5th instar	--	Abbassy et al. 1980
Diflubenzuron	Stable fly (Stomoxys calcitrans)	Topical 2 μg/fly	6 days +	97% of compound not metabolized 97% reduction of eggs hatch for 15 days	--	Spates and Wright 1980
Diflubenzuron 25W	Honey bees (Apis mellifera)	Aerial spray 8 applications 350 g a.i./ha	27 days to 7 mos	No significant difference after 7 mos; beneficial species apparently not affected.	Nontarget	Schroeder et al. 1980
Diflubenzuron 25 WP	Chrysopa oculata, Acholla multispinosa, Macrocentrus ancylivorus	Topical and contact with treated leaves and dietary	2 wks	Significant mortality C. oculata from topical and contact, but not through feeding; reduced emergence of M. ancylivarus.	Beneficial species	Broadbent and Pree 1984
Dimilin	Bacteria Oligochaetes Gastropods Mayfly Stonefly Diptera Coleoptera	0.1, 1.0, 10 or 50 μg/l into flowing stream (artificial)	5 mos	No effects on bacteria No effects on Oligochaetes No effects on Gastropods Mayfly affected at 1.0 μg/l Stonefly affected at 1.0 μg/l Dipterans affected at 10 μg/l Coleoptera unaffected	Nontarget	Hansen and Garton 1982

Appendix B (Concluded)

Formulation	Organism	Nature of Exposure	Time	Effects	Comments	Reference
Diflubenzuron	Honey bee (Apis mellifera)	Spray 0.20 and 0.40 kg a.i./tree (0.11 to 0.40 kg a.i./ha)	41 days	No reduction in adult or larval bees but small (0.11 to 0.30 μg/g) amount found in honey.	High dose Nontarget	Emmett and Archer 1980
TH 6040 paper impregnated 25% WP	Stable fly (S. calcitrans) Horn fly (Haematobia irritans)	Contact on paper and steer (0.5 to 0.012%) (0.28 to 0.005 mg/cm²)	4 hrs 2 to 5 days 7 days	LC_{50} for inhibition of egg hatch was 0.17%; 88% eggs did not hatch when flies exposed 7 days on steer; horn fly hatchability inhibited after 48 hour exposure.	No larval from manure	Wright and Harris 1976
Dimilin	Gypsy Moth	2 mist blower applications 0.0078 lb a.i./10 gal to 0.00025 lb a.i./10 gal	1 mo	Moth mortality 99.6 and 100% first spray inhibited larval parasite emergence.	Lasted 2 wks	Granett et al. 1976
Diflubenzuron (technical)	Barnacle (Balanus eburneus)	1 to 1,000 ppb immersion seawater	28 days	Dose dependent mortality; toxic over 28 days to fed group (200 ppb) and unfed group (400 ppb).	Intermolt deaths	Gulka et al. 1980
TH 6040 WP	Aquatic insects (62 species)	0.02 to 0.05 lb a.i./acre to pastures	3 to 4 days	Cladoceran and mayfly populations decreased but recovered; Corixid and notonectid nymphs slight mortality; beetles mostly tolerant; spiders no effects.	Nontarget	Miura and Takahashi 1975
Diflubenzuron	Soil mites 64 species	140 g a.i./ha aerial spray soil samples	2 wks to 30 mos after application	30 species showed significant changes; after 2 wks, 6 to 7 species reduced, however, 6 species increased.	Nontarget	Marshall 1979
Diflubenzuron (5% granules)	Heterostigmata, Cryptostigmata, Astigmata, Mesostigmata, Prostigmata	5 kg/acre on soil (0.25 kg a.i./acre)	2 to 45 days	60.1 ave. % reduction 44.1 ave. % reduction [illegible].6 ave. % reduction 40.8 ave. % reduction 58.5 ave. % reduction	Nontarget	Redman and AboKorah 1979

Appendix C

Summary of Diflubenzuron Toxicity Data for Fish

Appendix C

Summary of Diflubenzuron Toxicity Data for Fish

Formulation	Organism	Nature of Exposure	Time	Effects	Comments	Reference
Diflubenzuron 95% technical	Rainbow trout	Acute immersion in 15 l of solution 95% CI (mg/l)	96 hrs	LC_{50} >100	10 °C temp.	Johnson and Finley 1980
	Brook trout	Acute immersion in 15 l of solution 95% CI (mg/l)	96 hrs	LC_{50} >50	7 °C temp.	Johnson and Finley 1980
	Channel catfish	Acute immersion in 15 l of solution 95% CI (mg/l)	96 hrs	LC_{50} >100	22 °C temp.	Johnson and Finley 1980
	Bluegill	Acute immersion in 15 l of solution 95% CI (mg/l)	96 hrs	LC_{50} >100	20 °C temp.	Johnson and Finley 1980
	Yellow perch	Acute immersion in 15 l of solution 95% CI (mg/l)	96 hrs	LC_{50} >25	12 °C temp.	Johnson and Finley 1980
Diflubenzuron 25% WP	Cutthroat trout	Acute immersion in 15 l of solution 95% CI (mg/l)	96 hrs	LC_{50} = 57 (48-67)	12 °C temp.	Johnson and Finley 1980
	Rainbow trout	Acute immersion in 15 l of solution 95% CI (mg/l)	96 hrs	LC_{50} = 240 (201-286)	10 °C temp.	Johnson and Finley 1980
	Fathead minnow	Acute immersion in 15 l of solution 95% CI (mg/l)	96 hrs	LC_{50} >100	20 °C temp.	Johnson and Finley 1980
	Channel catfish	Acute immersion in 15 l of solution 95% CI (mg/l)	96 hrs	LC_{50} >100	22 °C temp.	Johnson and Finley 1980
	Bluegill	Acute immersion in 15 l of solution 95% CI (mg/l)	96 hrs	LC_{50} >100	20 °C temp.	Johnson and Finley 1980

Appendix C (Concluded)

Formulation	Organism	Nature of Exposure	Time	Effects	Comments	Reference
Diflubenzuron technical grade	Rainbow trout eggs and fingerlings	0.029 to 0.30 mg/l immersion flow through	30 days	No adverse effects	--	Johnson and Finley 1980
Dimilin 25% WP	Brown bullheads (Ictalurus nebulosus)	Control, 10, 100, and 500 ppm solutions	2 wks	No mortality	Problem with lab procedure and controls	Buckner et al. 1975
	Sunfish (Lipomis gibbosus)	Control, 10, 100, and 500 ppm solutions	2 wks	No mortality until 9th day	Problem with lab procedure and controls	Buckner et al. 1975
Diflubenzuron	Steelhead trout (Salmo gairdneri)	Acute flow through; Chronic flow through	96 hrs 9 wks	No effect $\leq$45 µg/l No effect $\leq$45 µg/l	Problem with lab procedure and controls	Hansen and Garton 1982
	Fathead minnow (Pimephales promelas)	Acute flow through; Chronic flow through	96 hrs 30 days	No effect $\leq$45 µg/l No effect $\leq$45 µg/l	Problem with lab procedure and controls	Hansen and Garton 1982
	Guppy (Lebistes reticulatus)	Acute flow through	96 hrs	No effect $\leq$45 µg/l	No chronic test	Hansen and Garton 1982
Dimilin WP-25	Rainbrow trout (S. gairdneri)	Chronic flow through	96 hrs	Statistical differences ($p \leq 0.025$) at 10 mg/l for glutamate oxalacetate transaminase serum value, dose related effect evident; no certain pathological significance.	May be stress response	Madder and Lockhart 1978
Diflubenzuron	Bluegill sunfish (Lepomis macrochirus)			LC_{50} = 135 mg/l	--	Madder and Lockhart 1978
	Saltwater minnow (Fundulus heteroclitus)			LC_{50} = 255 mg/l	--	Madder and Lockhart 1978

Appendix D

Summary of Diflubenzuron Toxicity Data for Birds

Appendix D

Summary of Diflubenzuron Toxicity Data for Birds

Formulation	Organism	Nature of Exposure	Time	Effects	Comments	Reference
Diflubenzuron	White Leghorn Chicken (hen)	Fed 10, 50, 100, and 500 ppm	8 wks	No adverse effects on feed consumption, body weight, egg production, egg weight, eggshell thickness, fertility, hatchability or progeny performance; very low accumulation; 5 wks after withdrawal none detectible in tissue.	--	Cecil et al. 1981
Dimilin TH 6040	Chickens	Fed 0, 2.5, 25, and 250 ppm	36 wks	No adverse effects on egg production, egg weight, eggshell weight, fertility, hatchability, progeny, percent hatch.	Hatchability greater for 25 ppm.	Kubena 1982
TH 6040	Chickens	Fed 1.6 to 50 ppm	16 wks	6.2 to 12.5 ppm level in feed completely reduced housefly larvae, but residues were found in eggs; at 3.1 ppm 63% larvae killed.	White eggs contained more residue.	Miller et al. 1975
Diflubenzuron TH 6040	Chickens	Fed 5 mg/kg in capsules	312 days	2-fold higher egg residue (0.4 ppm) in White Leghorns, rapid excretion, resistance to in vivo metabolism; in vitro <10% conversion to metabolites, 80% excreted within 12 days.	10 x normal dose	Opdycke et al. 1982
TH 6040	Chickens	Fed 10 ppm in ration	NA	Eggs accumulate Dimilin as well as in liver and fat, but no effect on fertility or hatchability.	White eggs contained more residue.	Miller et al. 1976

Appendix D (Concluded)

Formulation	Organism	Nature of Exposure	Time	Effects	Comments	Reference
TH 6040	Chickens	Fed to chicks	13 wks	Hens increased fat deposition and weighed twice that of controls; cocks did not appear to mature, dose-related decrease in testosterone	--	Smalley 1976
Dimilin 25 WP	3 species songbirds (insectivores)	Spray 1.2, 0.3, and 3.3 kg/ha	Nesting season	No significant effects on breeding or nestling growth.	Field study	De Reede 1982
Dimilin 25% WP	5 species warblers: *Mniotilta varia*, *Vermivora ruficapilla*, *Dendroica virens*, *D. fusca*, *Seiurus aurocapillus*; veery, *Hylocichla fuscescens*	350 g/ha spray	NA	No observable effect on avian population.	--	Buckner et al. 1975

NA - data not available.

Appendix E

Summary of Diflubenzuron Toxicity Data for Mammals

Appendix E

Summary of Diflubenzuron Toxicity Data for Mammals

Formulation[a]	Organism	Nature of Exposure	Effects	Comments	Reference
			Acute Oral Toxicity		
Dimilin	Rat (male and female)	NR	LD_{50} >4,640 mg/kg	Toxicity Category IV	Dobrowski and Lambert 1985 and U.S. Environmental Protection Agency 1987
Dimilin (25W)	Rat	NR	LD_{50} >40,000 mg/kg	--	Ag Chemicals 1983
Dimilin (technical)	Rat	NR	LD_{50} >4,640 mg/kg	--	Ag Chemicals 1983
Dimilin	Mouse (male and female)	NR	LD_{50} >4,640 mg/kg	--	Dobrowski and Lambert 1985
Dimilin (25W)	Mouse	NR	LD_{50} >40,000 mg/kg	--	Ag Chemicals 1983
Dimilin (technical)	Mouse	NR	LD_{50} >4,640 mg/kg	--	Ag Chemicals 1983
			Acute Intraperitoneal Toxicity		
Dimilin (technical)	Mouse	NR	LD_{50} >2,150 mg/kg	--	Dobrowski and Lambert 1985 and Wegler 1980
			Acute Dermal Toxicity		
Dimilin (25W)	Rabbit	NR	LD_{50} >10,000 mg/kg	Nonirritating	Ag Chemicals 1983
Dimilin (25W)	Rabbit	NR	LD_{50} >4,000 mg/kg	Toxicity Category III	U.S. Environmental Protection Agency 1987
Dimilin (technical)	Rabbit	NR	LD_{50} >2,000 mg/kg	--	Ag Chemicals 1983 and Wegler 1980

Appendix E (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	Reference
			Acute Eye Irritation		
Dimilin (25W)	Rabbit	NR	Nonirritating	--	Ag Chemicals 1983
Dimilin (technical)	Rabbit	NR	Marginal irritant	--	Ag Chemicals 1983
			Acute Inhalation Toxicity		
Dimilin (25W)	Rat	NR	LC_{50} >2.88 mg/l	Toxicity Category III	U.S. Environmental Protection Agency 1987
Dimilin (technical)	Rat	NR	LC_{50} >35 mg/l	--	Ag Chemicals 1983
			Subchronic Toxicity		
Dimilin (98% purity)	Rat (male Long Evans, 25 days old at the initiation of the study; 8 animals per treatment group, 15 animals per corn oil control group)	Daily exposure by intragastric intubation to 0, 15, 150, or 300 mg/kg in corn oil. Eight animals from each treatment group and 15 from the control group sacrificed at 14, 28, 42, or 90 days for histological and pathological examination.	Dose related decrease in plasma testosterone levels after 14 days of treatment, some decrease observed at 28 and 42 days, and no decrease observed at 90 days. Decrease in adrenal weight at all doses in the 28 day treatment group only. No difference between control and test groups with respect to testicular, prostatic, or body weights.	--	Patel and Santolucito 1980

Appendix E (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	Reference
			Subchronic Toxicity (Concluded)		
Diflubenzuron	Rat	Fed diets containing 3.125, 12.5, 50, and 200 mg/kg for 90 days.	NOEL = 50 mg/kg	Parameters measured	Wegler 1980
Diflubenzuron	Dog (Beagle, purebred)	Fed diets containing 20, 40, and 160 mg/kg for 90 days.	NOEL = 40 mg/kg. Observed marginal increases in methemoglobin and sulfhemoglobin at 160 mg/kg.	--	Wegler 1980
			Chronic Toxicity		
Diflubenzuron	Rat (males and females)	Fed diets containing 10, 20, 40, and 160 mg/kg for 2 yrs.	NOEL = 40 mg/kg Observed marginal increase in methemoglobin in both sexes at 160 mg/kg.	--	Wegler 1980
			Teratology, Reproduction, and Fertility		
Diflubenzuron	Rat	Fed diets containing 10, 20, 40 and 160 mg/kg daily for third generations.	NOEL = 160 mg/kg. No negative effects on growth or reproductive performance detected in parents or offspring for third generations. No adverse effect on mating, performance, pregnancy rate, duration of gestation, total litter loss, litter size, litter mean pup weight, pup mortality, and incidence of abnormalities.	--	Wegler 1980, Dobrowski and Lambert 1985
Diflubenzuron	Rat	Fed doses up to 100,000 ppm for one generation.	No teratological effects observed.	--	Dobrowski and Lambert 1985

Appendix K (Concluded)

Formulation	Organism	Nature of Exposure	Effects	Comments	Reference
			Teratology, Reproduction, and Fertility (Concluded)		
Diflubenzuron	Rat	Exposed orally to 1, 2, and 4 mg/kg days 6 to 15 of gestation.	No negative effects on growth or reproductive performance. No adverse effect on mating, performance, pregnancy rate, duration of gestation, total litter loss, litter size, litter and mean pup weight, pup mortality, and incidence of abnormalities.	--	Dobrowski and Lambert 1985
Diflubenzuron	Sheep (50 crossbreed Columbia-Rambouillet ewes, 3 Hampshire rams, 2 Dorset rams)	Fed breeding sheep 100 ppm for 7 to 8 mos, ending when lambs were weaned. Unsupplemented controls. After weaning, lambs fed 0, 12.5, 25, 100, or 250 ppm.	No affect on conception lambing rate, or birth weight. Live lambs/ewe averaged 1.55 for treated and 1.47 for untreated control. Lambs fed 0 to 250 ppm in diet after weaning had same daily weight gain food intake and food conversion. Feeding caused no change in RBC and WBC counts, hemoglobin BUN, protein, bilirubin and cell volume.	--	Escobar et al. 1980
			Carcinogenicity		
Diflubenzuron	Rat (males and females)	Highest dose - 10,000 ppm (500 mg/kg b.w.)	Not carcinogenic	--	U.S. Environmental Protection Agency 1987
Diflubenzuron	Mouse (males and females)	Fed diets containing 4, 8, 16, and 50 mg/kg for 80 weeks.	NOEL = 50 mg/kg. No change in spontaneous tumor profile.	Parameters for NOEL unspecified.	Maas 1980

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[a] Dimilin (25W) - 25% Wettable Powder

NR - not reported, RBC - red blood cell, WBC - white blood cell, BUN - blood urea nitrogen.

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Appendix F

Summary of Neurotoxicty and Behavior Data for Diflubenzuron

(No data currently available)

Appendix G

Summary of Diflubenzuron Mutagenicity Data

Appendix G

Summary of Mutagenicity Data for Diflubenzuron

Formulation	Organism	Nature of Exposure	Effects	Comments	Reference
Diflubenzuron (technical)	*Salmonella typhimurium* (TA98, TA100, TA1535, TA1537)	Dosed at 10, 100, or 1,000 μg/plate with and without metabolic activation.	Not mutagenic; no increase over spontaneous reversion frequency.	Positive control (aflatoxin B_1) included to confirm activity of metabolic activation system.	MacGregor et al. 1979
Dimilin W-25 (25% wettable powder)	*S. typhimurium* (TA98, TA100, TA1535, TA1537)	Dosed at equivalent to 19, 189, or 1,860 μg diflubenzuron/plate with and without metabolic activation. Reverse point mutation assay.	Not mutagenic; no increase over spontaneous reversion frequency.	Positive control (aflatoxin B_1 included to confirm activity of metabolic activation system.	MacGreger et al. 1979
Diflubenzuron	*S. typhimurium* (TA98, TA100, TA1535, TA1537, TA1538)	Dosed at 1,000 μg/l plate with metabolic activation. Reverse activation. Reverse mutation assay.	Not mutagenic; no increase over spontaneous reversion frequency.	Six proposed metabolites of diflubenzuron (4-chloroaniline, 4-chlorophenylurea, 4-chlorophenol, acetanilide, 4-chloracetanilide, and 2,6-difluorobenzoic acid) were also tested at various concentrations in these 5 bacterial tester strains. No increase over spontaneous reversion frequency was observed for any of these compounds. 2,6-difluorobenzuric acid produced toxic effects in TA1537 that were mistaken for an increase of mutagenicity.	Seuferer et al. 1979

Appendix G (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	Reference
Diflubenzuron	Mouse lymphoma cells *in vitro*	Dosed at 1.17, 4.69, 18.75, 37.5, 75, 150, and 300 μg/ml with and without metabolic activation. Forward mutation assay.	Not mutagenic; no increase over spontaneous mutation frequency.	Assay tests for loss of function of thymidine kinase enzyme. Positive control (dimethylnitrosamine) included to confirm activity of metabolic activation system. Plating efficiency was not decreased at any dose.	MacGregor et al. 1979
Diflubenzuron	Mouse (male Swiss-Webster)	Dosed at 15, 150, and 1,500 mg/kg by gavage (5 animals per group). Micronucleus test.	Not mutagenic; no increase over control in the frequency of of micronuclei or the the ratio of mature to polychromatic erythrocytes in bone marrow.	Assay tests for chromosome loss or breakage resulting from mitotic abnormalities in developing erythrocytes in bone marrow. However micronucleus test is not sensitive to increases in the incidence of micronucleated erythrocytes of less than 3 to 4 times background. The positive control, triethylenemelamine produced a statistically significant increase in micronucleus frequency.	MacGregor et al. 1979

Appendix G (Concluded)

Formulation	Organism	Nature of Exposure	Effects	Comments	Reference
Diflubenzuron	Hamster (10 day pregnant females)	Dose intraperitoneally and 50 mg/100 g body wt. Host-mediated transplacental assay.	Not mutagenic; no increase over control in the frequency of of transformed colony formation or efficiency of formation in soft agar of fetal cells.	Assay tests for frequency of morphological transforma- and efficiency of growth in soft agar (thought to be two characteristics of preneoplastic cells) of hamster fetal cells following *in utero* exposure to mutagens. Three positive control mutagens did increase frequency of morphological transformation and efficiency of growth in soft agar in this assay. Assay has not yet been validated for the detection of mutagenic substances.	Quarles et al. 1980
Dimilin	Mouse	Dosed at 100, 500, 2,500, and 5,000 mg/kg.	Increased number of chromosome aberrations as detected by metaphase analysis.	No dose-response relationship demonstrated, and is therefore, not sufficient to prove that Dimilin is mutagenic.	Kurinnyi et al. 1982

Appendix H

Summary of Metabolism Studies with Diflubenzuron

Appendix H

Summary of Studies on Metabolism and Diflubenzuron

Formulation	Organism	Nature of Exposure	Time	Effects	Comments	Reference
Diflubenzuron	*Rattus norvegicus*	31 ppm to rat C6 astrocytoma cell culture	NA	No measurable effect on cell morphology or division; no inhibition of glycosaminoglycans production; inhibition of N-acetylhexosaminyltransferases.	*In vitro*	Stoolmiller 1978
Diflubenzuron (Dimilin)	Rat	4 mg/kg to 900 mg/kg	3 days	Excretion almost complete 72 hrs after dosing; intestinal absorption decreases with increased dose; 80% unchanged in body.	--	Willems et al. 1980
Diflubenzuron (technical)	Swine	Oral dose	11 days	82% in feces and 5% of dose in urine and 0.43 ppm to gallbladder.	All tissues analyzed	Opdycke et al. 1982
TH 6040	Dairy cows	Fed 0.25 to 16 mg/kg	4 to 5 months	Little to no residue in milk; maximum residue found in fat 0.25 ppm; half-life in manure 4 to 5 days; no adverse effects.	--	Miller et al. 1976
Diflubenzuron (TH 6040) 25% WP	Cattle, sheep, and flies	Single oral dose by tube 10 mg/kg	Sampling at 24 hrs	Highly biodegradable, residues are rapidly metabolized by cattle and sheep but much less so by the stable fly and horsefly.	*In vivo*	Ivie 1978
Dimilin	Stable fly (*Stomoxys calcitrans*) House fly (*M. domestica*)	Topical 2 µg/fly	4 days	35 to 50% detectable in excreta; diflubenzuron quite resistent to metabolism (>97% by stable fly).	--	Ivie and Wright 1978

Appendix H (Continued)

Formulation	Organism	Nature of Exposure	Time	Effects	Comments	Reference
Diflubenzuron	Housefly (*Musca domestica*)	Injected 2684 dpm/ fly	72 hrs	Conjugation in major metabolic pathway of diflubenzuron; 74% remains unchanged and 16.5% into excreta.	--	Chang 1978
Diflubenzuron	Boll weevil (*Anthonomous grandis*)	Injected 4 µg/weevil	4 days	Conjugation in major metabolic pathway; 57.7% recovered and 25% from excreta.	--	Chang and Stokes 1975
TH 6040 (Dimilin W-25)	Boll weevil (*A. grandis*)	Dipped in or injected or fed 120 ng/weevil	44 hrs	50% excreted; no data to indicate weevil could metabolize appreciable amount of diflubenzuron.	--	Still and Leopold 1978
Difluenbzuron	Boll weevil (*A. grandis*)	Oral and topical	22 days	Hatch inhibited by about 80% for 10 days; at 50% hatchability eggs contained 0.6 ppm, female regained fertility.	Target	Bull 1980
Diflubenzuron >95% pure	*Pieris brassicae* (Lepidoptera)	Atomized first 3 days of 5th instar	3 days	Diflubenzuron diffuses to the whole tissue, but action in epidermal cells; slight poison in other cells; present in other tissues (fat bodies) related to general metabolic effects and detoxification.	Male versus female differences noted.	Truchet et al. 1981
Diflubenzuron	*Orgyia pseudotsugata*, *Choristoneura occidentalis*	18 µg/needle fed to larvae	48 hrs	86 to 90% passed through body without being absorbed; toxicity related to the amount retained.	Target	Granett et al. 1980
Diflubenzuron >99% pure	2-spot spider mite (*Tetranychus urticae*), Lima bean (*Phaseolus lunatus*	Metabolism chamber precoated with labeled DFB	1 to 96 hrs	Absorbed and metabolized slowly by mites but not bean plants (topical); plants metabolized injected diflubenzuron.	*In vivo*	Franklin and Knowles 1981

Appendix H (Concluded)

Formulation	Organism	Nature of Exposure	Time	Effects	Comments	Reference
Diflubenzuron	4 eukaryotic microorganisms	Incubated room temperature	6 to 20 days	Rapidly degraded by soil fungi, lethal to *Salmonella*.	*In vitro*	Seuferer et al. 1979
Diflubenzuron 99% pure	Culture Harding-Passey Melonoma cells	Preincubated culture 1 hr, then incubated for 15 mins	<5 mins	Significant inhibition uptake of uridine, adenosine, cytidine.	With DMSO	Mayer et al. 1984
Diflubenzuron 99% pure	Mammal cells	16.14m Ci/m mol labelled in cultured C6 glial rat cells *in vitro*.		Not cytotoxic nor did it inhibit synthesis of complex carbohydrates; 98% recovered.	With DMSO	Bishai and Stoolmiller 1979

NA - data not available.

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